GENETICS

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BEARING ON HEREDITY AND VARIATION

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LETHAL ACTION OF THE GENE FOR EXTENSION OF BLACK PIGMENT IN THE FOWL[1]

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Received July 18, 1950

THE solid black plumage that is characteristic of several breeds and varieties of the domestic fowl results from the combined action of the genes *C*, for production of melanic pigment, and *E*, for its extension to all parts of the plumage. Birds of the genotype *cc* (whether *EE* or *ee*) are recessive whites, and those with *CC ee* have their black pigment restricted to the neck, wings, and tail. This latter phenotype, which is called Columbian restriction, is most evident in such birds as Light Sussex, Light Brahmas, and the Columbian varieties of several breeds, but it is also characteristic, though less recognizable, in many gold breeds and varieties. These include Rhode Island Reds, New Hampshires, and the buff varieties of several breeds.

The studies reported hereunder show (1) that *E* is lethal to some of the black chicks when they carry no genes for patterns in their downy plumage, but (2) that this lethal action is escaped by black chicks carrying the sex-linked gene *B*, which later causes barring in the feathers of growing birds and of adults. These peculiar effects apparently result from the facts that *E* shortens the plumules that comprise the first downy plumage of the chick, and that *B* prevents such shortening or lessens it.

During the 42 years that have elapsed since SPILLMAN (1908) elucidated the genetic basis for barring, much information has accumulated about the expression of *B* in males, in females, and in heterozygotes, about its autonomous action in transplanted cells, and about its interaction with other genes. A recent review of this subject elsewhere (HUTT 1949) makes detailed discussion unnecessary here. It will suffice to remind the reader that in the downy plumage of the chick *B* is manifested—not as barring—but as an irregular white spot in the occipital region of the head. It also causes some reduction of melanin throughout the down but this effect is most evident in homozygous males, which usually have somewhat lighter, greyer down than the hemizygous females. This difference in color between the sexes is enhanced when the *BB* ♂ ♂ and *B*– ♀ ♀ carry some additional gene that also reduces pigment in the down. Recognition of this fact by PUNNETT and PEASE (1930) led to their production of the Cambars, the first in a succession of auto-sexing breeds in which the sexes may be distinguished at hatching by color of the down.

[1] Number 24 in the author's series entitled "Genetics of the Fowl."

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Sex of the chicks can also be recognized with complete accuracy in the offspring from any cross of barred ♀ × non-barred ♂, and several millions of such chicks are hatched annually in the United States. The cross is very popular, not merely because it permits the poultryman to buy either males or females according to his preference, but also because in the two breeds most commonly crossed there are available many strains that have been bred for high egg-production, for large eggs, and for other characters of economic importance. These breeds are the Barred Plymouth Rock and the Rhode Island Red. They have been differentiated as distinct breeds for so many years that when they are crossed a certain amount of hybrid vigor is usually evident. Since this improves the hatchability of the eggs, the growth and viability of the chicks, and sometimes, also, the productivity of the adults, the cross is deservedly popular with both hatcherymen and their customers.

In May of 1945 the author was asked by MR. STEPHEN S. WALFORD of HALL BROTHERS HATCHERY, INC., Wallingford, Connecticut, to account for certain chicks from this cross that were unsalable because they lacked the normal covering of down on the back. The fact that these bare-backed chicks were always females and that flocks and hatches producing them yielded only rarely or never a similarly defective male chick suggested an intriguing genetic problem. This it has proven to be.

DESCRIPTION OF BARE-BACKED FEMALES

Among many bare-backed chicks examined during this investigation, the defective condition was extremely variable. Some of them show no sign of feathers in the region overlying the sacrum (fig. 1, right). Others have some down in this area, but it is short, and sometimes matted or curled (fig. 1, left). These abnormalities are most pronounced in the posterior-spinal feather tract, and particularly toward the sides and posterior portion of that tract. Because the areas with defective down adjoin the apterium (space with few follicles or none) between the posterior-spinal tract and the femoral tract, the general appearance is that of a large U-shaped bare area, which conforms to the shape of the back (figure 1). In normal chicks the comparatively bare area of the apterium is hidden by the branching downy plumules at the borders of the spinal and femoral tracts. With short down, or none at all, this space between the feather tracts stands out distinctly.

Some of the bare-backed chicks show scars on the skin as if the latter had been torn away in spots. These extend through the areas that lack down, or have only short down, but are not found in chicks with normal long down. Some chicks have bits of shell stuck to the bare areas.

In many of the bare-backed chicks, the down appears short right up to the head. There is a continuous gradation between the most extreme cases and chicks that are normal. Some that cannot be classified as bare-backed show at hatching a somewhat wiry, sticky, flattened down (fig. 2), but 24 hours later, when the down has fluffed out, most of these are almost indistinguishable from normal chicks. Long before maturity, all the bare-backed

chicks—even the most extreme cases—have normal plumage. Most of them appear normal as soon as they get their first juvenile plumage that replaces the down.

Inquiries revealed that in other hatcheries these bare-backed chicks are well known. Their frequency varies in different stocks, and reputedly in different hatches from the same stock, but also according to the view of the individual who makes the arbitrary distinction between those too bare to sell and those that are not. From figures supplied by four hatcheries, it would appear that the average incidence of "bare-backed culls" among the black females hatched from this cross is from 0.75 to 1.0 percent, but that it may run as

FIGURE 1.—Chicks showing short, matted down, with partial bareness (left) and complete absence of down in the sacral region (right).

high as 3 percent in some stocks. All reports agreed that these abnormal chicks are not found in pure Barred Plymouth Rocks, in pure Rhode Island Reds, or in other common breeds, but almost without exception only among females from the cross Barred Rock ♀ × Rhode Island Red ♂. The exceptions to this rule are the few bare-backed males seen at rare intervals. It seemed unlikely, therefore, that the abnormality could be attributed to some simple mutation.

GENETIC ANALYSES

In addition to the *B* and *b* alleles, another pair of sex-linked genes segregates in the cross under consideration. These are *S* (silver) and *s* (gold).

Since their effects are less evident in chicks with extended black (*EE* or *Ee*) than are *B* and *b*, the latter are used for identification of sexes, rather than *S* and *s*. The original cross and progeny are as follows:

Barred Rock ♀ × Rhode Island Red ♂ →	Non-barred ♀	+ barred ♂
EE BS/– *ee bs/bs*	*Ee bs/–*	*Ee BS/bs*

To determine the genetic basis for bare-backs, matings were arranged so that eventually sons of bare-backed females could be back-crossed to other bare-backed females, with genotypes such that in the resultant chicks the two

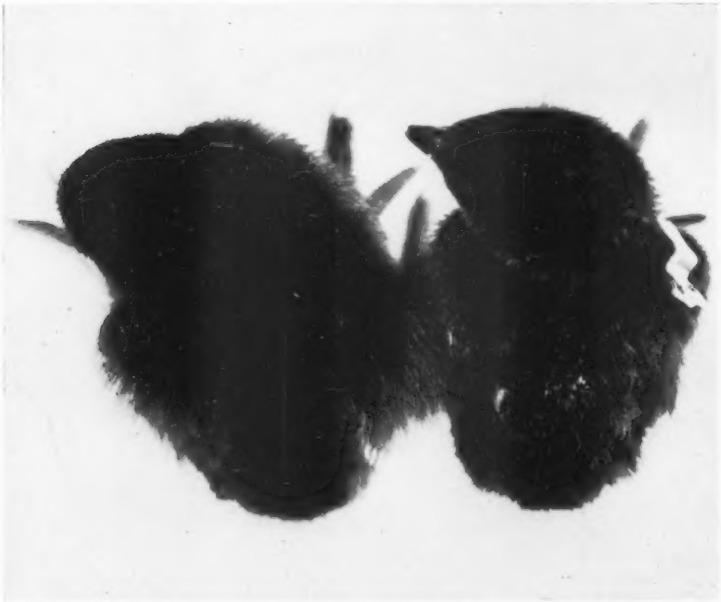

FIGURE 2.—Normal chick (left) and one with short, wiry down but no bare areas (right).

alleles in each of the three pairs of genes affecting color—*E*, *B*, and *S*—would segregate in the ratio of 1 : 1. Any possible relation of the deficiency of down to barring or non-barring, to extension or restriction of black, and to silver or gold, should be fully revealed by such a mating.

To that end, in 1946 three once-bare F_1 females from the original cross shown above were mated with a male of the genotype *Ee BS/bs*. This yielded 36 chicks—none with bare backs. From these, when they matured, there were selected for subsequent matings males of the genotype *ee BS/bs*. (Barred and silver birds of this type can be identified in those with Columbian restriction at maturity, but not at hatching.) Another step in preparation for the critical test matings was to obtain, in the spring of 1946, a good supply of bare-backed

chicks and some normal ones, all being F_1 females from the cross, Barred Rock ♀ × Rhode Island Red ♂. These were kindly provided by MR. M. C. BABCOCK of Ithaca.

In 1947, two of the *ee* males heterozygous for *B* and *S* were each mated (in separate pens) with 14 of these F_1 females. Each breeding pen contained 9 or 10 hens that had been bare-backed when hatched and 4 or 5 that had had normal down. Results of these matings are shown in table 1.

In table 1 the data are not given separately for males and for females because the two sexes could not be differentiated at hatching. Whether extended or restricted, barred or non-barred, silver or gold, males and females were to be expected in approximately equal numbers in each phenotype, and classifications at later ages agreed with that expectation. The incidence of bare backs in the two sexes is considered later.

TABLE 1

Segregation of colors and bare-back in chicks hatched from the mating of ♂♂ ee BS/bs × ♀♀ Ee bs/–.

Sire and dams	Extended black (*Ee*)				Restricted black (*ee*)			
	Barred *Bb* or *B–*		Non-barred *bb* or *b–*		Silver *Ss* or *S–*		Gold *ss* or *s–*	
	Normal	Bare	Normal	Bare	Normal	Bare	Normal	Bare
♂ A × 8 bare ♀♀*	24	4	15	11	40	0	24	0
♂ A × 5 normal ♀♀*	16	0	14	7	24	0	16	0
♂ B × 10 bare ♀♀*	33	2	26	16	37	0	50	0
♂ B × 4 normal ♀♀*	15	0	11	3	11	0	17	0
Totals	88	6	66	37	112	0	107	0
	94		103					
	197				219			

* With bare back or normal down as chicks.

When considering the data in table 1, it should be understood that, although the barred and non-barred chicks were classified as such only in those showing extended black, these same two classes occurred in the restricted chicks, but were unrecognizable in the latter. Similarly, about half the chicks with extended black were silver and the other half gold, but these two types cannot be differentiated with complete accuracy in the black chicks. The totals show close fits to the expected ratios of 1 barred : 1 non-barred, 1 silver : 1 gold, and 1 extended : 1 restricted black. For this last-named segregation (197 : 219), which shows the greatest deviation from expectation, $\chi^2 = 1.162$, P = .2 – .3.

On appraising the results of these matings, one significant finding is that not one of the chicks with restricted black showed a bare back. This eliminated any possibility that the defect might be attributed to some recessive sex-linked gene contributed (in the original cross of Barred Rock ♀ × Rhode Island Red ♂) by the male. In such an event, all of the gold chicks from bare-backed

dams should have shown the defect because their *s* genes came, either through their dam or through their bare-backed paternal grand-dam (or, in the case of males, through both), from a Rhode Island Red male that had sired bare chicks.

Considering next only the chicks with extended black, the data are best evaluated by combining the results for the two pens and for the two kinds of dams. When this is done, the frequencies of bare-backed chicks were as follows:

	In non-barred progeny	*In barred progeny*
From 18 bare dams	39.7%	9.5%
From 9 normal dams	28.6%	0.0%

It is clear that the defect is primarily one associated with non-barred, extended black. This is consistent with the reports of hatcherymen that the bare-backs are almost invariably females, and that males with the defect are very rare. In their chicks from this sex-linked cross the females are always *b*–, whereas the males are *Bb*. While 6 of 94 barred chicks did show the bare back (table 1) these came only from hens that had been bare as chicks. Moreover, these hens were mated with sons of bare-backed dams. Since selection of this sort tending to intensify the abnormality is exactly the reverse of what happens in practice, the production of barred and bare chicks in these experimental matings does not controvert the evidence that ordinarily *B* prevents that abnormality from occurring.

Apart from the remarkable influence of the gene *B* in promoting normal development of down, it is evident that bare-backed chicks come more frequently from bare-backed dams than from normal ones. Since there is little indication of any Mendelian segregation, it seems probable that manifestation of the abnormality depends in part upon multiple factors or modifying genes. The incidence of bare-backs among the total of 103 non-barred black chicks hatched in these matings was 36 percent. The fact that it exceeded by so much the usual frequency of 0.5 to 3.0 percent reported by hatcherymen is attributable (at least in part) to the concentration of genes tending to enhance the abnormality in these matings. Differences between the writer's classification and that of hatcherymen may also have been a factor.

Sex was eventually determined for 32 of the 43 bare-backed chicks. Among 28 non-barred chicks there were 19 ♀ ♀ and 9 ♂ ♂; among 4 barred chicks there were 3 ♀ ♀ and 1 ♂. The finding that a considerable number of male chicks could be bare-backed showed that the abnormality is not sex-limited, but there is some indication that even in black chicks genetically alike, whether barred or not, the defective down occurs more frequently in females. Since eleven of the bare-backs were lost on the rearing range without determination of their sex, a clear answer to this question was not available from these chicks.

Altogether, the data of 1946 and 1947 showed (1) that the abnormality is neither sex-linked nor sex-limited, (2) that it is not caused by any unifac-

torial mutation, (3) that it is not found in gold and silver chicks with Columbian restriction of black, and (4) is most frequent in non-barred chicks with extended black, but (5) can appear in some of the barred chicks that have a concentration of genes tending to induce the bare-back condition.

RELATION OF COLOR TO LENGTH OF DOWN

The barest chicks seemed to have the shortest down. Some black chicks also had short down but could hardly be classified as bare. Between these two types there was a continuous gradation in the degree of bareness, and apparently a corresponding gradation in the length of down. Evidence that the down is shorter in black chicks than in those with blue, dominant white, or restricted black had previously been reported by KABYSTINA and PETROV (1935). It seemed probable, therefore, that in chicks with extended black the down is shorter than in those of other colors, and that the bare-backed ones represent the extreme variants.

Matings were therefore arranged in 1948 to provide chicks for measurements of length of down in full siblings that differed only by the two genes comprising one pair of alleles. These matings yielded siblings either *E* or *e*, *e S* or *e s*, *E B* or *E b*. Samples of down were plucked on the day after hatching. To determine whether the short down of bare-backed chicks represented a localized shortening of the feathers or a general one affecting all regions of the body, the samples of down were taken, in nearly every case, from the top of the head, the lower back, and the tip of the wing of each chick. The number of plumules taken was never counted when they were plucked, but was usually well over 10 per sample.

To permit measurements of maximum length, each plumule was soaked in water and drawn out in a straight line on a microscope slide. When 10 had been thus arrayed in a compact row, the slide was placed on the stage of a delineascope and the images were projected by the overhead mirror to a screen. This was placed at such a distance as to give a magnification of exactly 10 diameters. It consisted merely of a sheet of white paper fastened on the back of a glass door, so that the images could be measured on the back of the screen. Uniform measurements were assured by clamping both screen and delineascope in fixed positions, and by marking on the stage of the delineascope a constant position for all slides.

Measurements were taken to the nearest millimeter; hence a measurement of 12.8 cm represented an actual length of 12.8 mm. By measuring 10 feathers per sample (except in the few cases where 10 were not available) the average length for the sample was quickly determined to the nearest 0.1 millimeter. Measurements of approximately 2,970 feathers are summarized in table 2. In appraising these data, it should be remembered that each of the 18 mean lengths given is determined from measurements of feathers to the number of ten times the corresponding number of chicks.

It is evident from inspection and from the values of P (in *t* tests for significance of the differences) that (1) among chicks with Columbian restric-

TABLE 2

Mean lengths of down feathers from chicks of different colors.

Phenotype		Chicks, number	Mean length (in mm) ± standard error		
Down	Genes		Head	Back	Wing
(1) Restricted, silver	*ee S*	19	9.34 ± 0.47	12.92 ± 0.38	14.90 ± 0.42
Restricted, gold	*ee s*	23	8.92 ± 0.35	12.18 ± 0.22	14.70 ± 0.35
Difference	....	....	.42	.74	.20
t	....	....	0.715	1.695	0.371
P	....	....	.48	.10	.71
(2) Restricted, non-barred, gold	*e b s*	14	8.72 ± 0.29	13.33 ± 0.23	15.01 ± 0.61
Extended, non-barred, gold	*E b s*	21	6.03 ± 0.27	9.25 ± 0.36	11.20 ± 0.44
Difference	....	....	2.69	4.08	3.81
t	....	....	6.750	9.499	5.068
P	....	....	<.001	< .001	< .001
(3) Extended, barred	*Ee B*	6	8.78 ± 0.35	11.85 ± 0.17	12.42 ± 0.79
Restricted	*ee*	16	8.65 ± 0.35	12.15 ± 0.42	13.94 ± 0.53
Difference	....	...	.13	.30	1.52
t	....	....	.261	1.242	1.607
P	....	....	.80	.23	.13

TABLE 4

Comparisons of barred and non-barred chicks (all extended black) with respect to length of down on the back.

Kind of chicks	Barred		Non-barred		Difference	*t*	P
	Number	Mean length, mm	Number	Mean length, mm			
(1) Siblings	16	12.47 ± 0.30	16	10.46 ± 0.32	2.01	4.55	< .001
(2) General population	35	11.38 ± 0.14	35	9.86 ± 0.20	1.52	6.24	< .001

tion, there is no significant difference in length between silver and gold feathers. However, (2) among siblings, all of which are non-barred and gold, those with extended black have shorter feathers than the ones with Columbian restriction. The differences in the head, back and wing of 2.69, 4.08, and 3.81 mm, respectively, are all highly significant statistically. Finally, (3) in chicks with extended black that carry the gene *B*, the down is not significantly shortened by *E*, but is almost as long as in siblings with restricted black.

From the mean lengths of feathers in different regions of non-barred chicks with extended or restricted black (table 2) it would appear that the gene *E* shortens the down more on the back (4.08 mm) than on the head and wing tip. However, the proportional reductions in these three areas are 30.6, 30.8, and 25.4 percent, respectively. It seems clear, therefore, that the shortening induced by *E* is effective throughout the entire plumage, and is no greater in the back than elsewhere. The fact that chicks with extremely short down seem barest on the back might result from the feather follicles being fewer per unit of area there than in some other regions, particularly the head, where they are close together. The bare effect on the back is also enhanced by the bilateral apteria between the posterior spinal feather tract, and the femoral ones.

Further evidence that the effect on the length of down is uniform, at least in the three areas sampled, and presumably over the entire body, is provided by the high correlation between mean length of down in any two of these three areas in the same bird. This applies equally to chicks with Columbian restriction and to those with extended black (table 3).

TABLE 3

Correlations between mean length of down in different regions of the same chick.

Regions compared*	Restricted black (silver and gold)		Extended black (non-barred)	
	Chicks, number	Coefficient of correlation	Chicks, number	Coefficient of correlation
Head and back	42	+ .979	21	+ .982
Head and wing	42	+ .987	21	+ .975
Back and wing	42	+ .989	21	+ .980

* Ten feathers from each region of every chick.

With respect to the influence of the gene for barring on length of down, the data in table 2 show only that when *B* is added to a genotype containing *E*, the feathers are not significantly shorter than in *ee* chicks with restricted black. The assumption from this was that *B* offsets the shortening tendency of *E*, and hence that among chicks all showing extended black, those carrying *B* should have longer down than the non-barred ones. To test that hypothesis, further samples of down were measured. Some of these came from 32 full siblings, all of them black, but half being barred (as attested by the white spot on the head) and half not. Other samples were taken from a general population of 70 chicks from the cross Barred Rock ♀ × Rhode Island Red

♂. Down was plucked from the middle of the back on the day after hatching and 10 feathers per chick were measured, as before. The data are given in table 4.

As had been anticipated, the non-barred chicks had shorter down than the barred ones, the difference being 2.01 mm in one lot of chicks and 1.52 mm in the other. Both of these differences were shown by the *t* test to be statistically highly significant. A valid criticism of the data from the general population is that, since these chicks came from a sex-linked cross, all the barred birds were males and all the non-barred ones females. This was answered later by testing for any effect of sex on length of down in chicks all black and non-barred. The results showed no significant difference between the sexes in this respect. A greater length of barred than of non-barred down was also evident in the data of KABYSTINA and PETROV (1935), but the difference (0.66 mm) was apparently not significant in their material.

These studies of the relation of color to length of down confirm the evidence from the genetic analyses that the bare-backed black chicks from the cross of Barred Rock ♀ × Rhode Island Red ♂ are the extreme variants among chicks of the genotype *CC Ee bs/–*, in all of which the down is shortened by the gene *E*. The fact that the male chicks from this cross are seldom affected is attributable to their being of the genotype *CC Ee BS/bs*. In their case, *B* prevents any great shortening of the down by *E*. The fact that the females carry *s* and the males *S* is of no significance as these two alleles were proven to have no effect on the length of down.

The ability of *B* to counteract the shortening tendency of *E* undoubtedly results from the reduction of melanin induced by the former. It is probable that the maximum influence of the barring gene on length of down is not revealed by the data in tables 2 and 4, for none of the barred chicks there considered were homozygous for *B*. In adult homozygotes, there is less melanin in the plumage and skin than in birds of the genotypes *Bb* and *B–*. It would not be surprising if the same difference in chicks should cause the homozygous barred males to have still longer down than heterozygotes and females.

LETHAL ACTION OF THE GENE *E*

A. *Evidence from unhatched chicks*

In 1947 and 1948, during routine examination of eggs that failed to hatch, it was noticed that among chicks dying during the last three days of incubation, or still alive at 22 days, the proportion of black non-barred ones with bare backs was higher than in their black, non-barred siblings that hatched. In these dead chicks, the most consistent abnormality was not the large bare area seen in some of those that hatch, but rather the very short down, often so knotted that the skin seemed sparsely covered with pebbly black granules. In many of these bare chicks the abnormal down was evident, not only over the back, but also right up to the top of the head and even on the wings (fig. 3). This same "clubbed" down is commonly seen in chick embryos that have died from a deficiency of riboflavin in the egg.

This abnormality was so marked as to suggest that it might be in some cases the cause of death. Moreover, it was found (in these matings) only in the black, non-barred chicks. Although bare-backed chicks were consistently less frequent among those that hatched than in those that failed to do so (table 5) the difference was not statistically significant in the limited material available.

To get the large number of chicks and dead embryos necessary for a conclusive answer to this question, arrangements were made with a nearby hatchery which produced weekly several thousand chicks from the cross

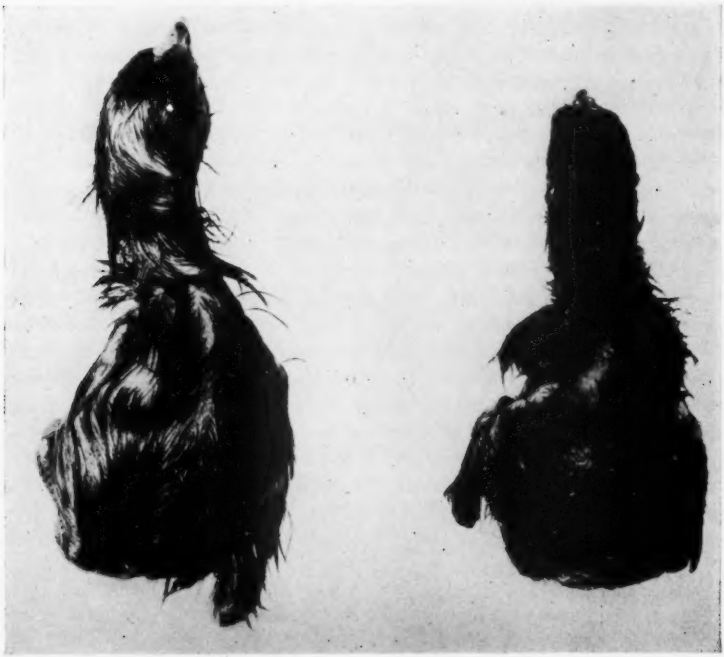

FIGURE 3.—Chicks that died at 19–21 days of incubation showing (left) a barred male with normal down and (right) a non-barred female with bare back and short granular down on the back, head and wings.

Barred Rock ♀ × Rhode Island Red ♂. From the hatches of two successive weeks (May 17 and 24, 1948), MR. MONROE BABCOCK kindly provided (1) all the eggs that failed to hatch, (2) records of the numbers of male and female chicks that did hatch, and (3) a record of the number of chicks that he classified as bare-backed. These last included only those that were culled out as being too bare to sell. Because the male and female chicks from this cross can be distinguished with complete accuracy at hatching by the headspot of the males, there can be no question of the accuracy of these data. In this case, the hatcheryman was particularly careful because of his own interest

TABLE 5

Frequencies of chicks with normal down or bare-backed among black, non-barred chicks that hatched and embryos that died at 19 to 22 days of incubation.

Year	Hatched chicks		Dead embryos		χ^2	P
	Normal down	Bare-backed	Normal down	Bare-backed		
1947	66	37	11	13	2.735	.098
1948	11	11	4	16	2.898	.091

in the investigation. Moreover, the fact that his customers paid about three times as much for female chicks as for males necessitated strict accuracy in classifying the chicks according to sex.

The unhatched eggs were obtained from the hatchery about 6 hours after termination of the normal 21-day period of incubation, and were broken individually for examination within 6 hours of their receipt. This process was simplified by first candling all the eggs and removing those thus found to be infertile or to contain embryos that had died at early stages of incubation. When the eggs were broken, 215 embroys were eliminated from the records because they were not developed far enough to permit accurate determination of sex by the presence or absence of a well-defined head spot. This took out most of the embryos dead at 15 days or earlier. It left a total of 1213 that died after that age and 69 that managed to hatch after their receipt from the hatchery. The incidence of bare-backed chicks among all of these, and among the chicks that hatched on time at the hatchery, is shown separately for each sex in table 6.

The sex ratio of 7,344 ♂♂ : 7,329 ♀♀ among all chicks, whether hatched or dead, was remarkably close to the equality of numbers expected. However, among the chicks that hatched on time, there was a deficiency of 265 ♀♀ below the number of males hatched. This number was matched closely by the

TABLE 6

Distribution of bare-backed chicks among 14,673 that hatched or died after fifteen days of incubation, all from Barred Rock ♀♀ × Rhode Island Red ♂♂.

Age group	Males, *Ee BS/bs*		Females, *Ee bs/—*		Deficiency or excess of females
	Normal down	Bare	Normal down	Bare	
(1) Hatched on time	6,828	0	6,510	53	−265
(2) Hatched late	30	0	31	8	+ 9
(3) Alive, or pipped	203	3	191	111	+ 96
(4) Unpipped, dead after 15 days	280	0	285	140	+145
Sub-totals for (2)+(3)+(4)	(513)	(3)	(507)	(259)	(+250)
Grand totals	7,341	3	7,017	312	
	7,344		7,329		− 15

highly significant excess ($P = < .001$) of 250 ♀ ♀ among the 1282 chicks that died or hatched late. These, in turn, could be accounted for entirely by the 259 bare-backed females that failed to hatch on time. It should be noted that deaths of chicks with normal down were 483 among the males and 476 in the females. With respect to mortality from non-specific causes, such an equality of the sexes among embryos dying late in incubation was to be expected from earlier studies by various investigators (*v. infra*). Only 3 males could be classified as bare-backed. This accentuates the fact that the deficiency of females hatching on time can be fully accounted for by the bare-backed females that died during the later stages of incubation.

Considering only the 1213 chicks that failed to hatch (and excluding the 69 that hatched late), the proportion of males among them was only 40.1 percent. This is much lower than the corresponding percentages of 47.56 found by BYERLY and JULL (1935), of 48.59 in previously-reported data summarized by them, and of 49.2 found by ASMUNDSON (1941) in chicks and embryos that failed to hatch. It is probable that most of these data pertain to pure breeds, although in some cases the breeds were not specified. However, the marked excess of dead females in the present writer's material cannot be ascribed to some peculiar effect of sex-linked crosses in general, for CREW (1938) found the proportion of males to be 51.03 percent in unhatched chicks from sex-linked crosses other than the one under consideration in this paper. The only previous data known to the writer for the cross, Barred Rock ♀ × Rhode Island Red ♂, are those of LANDAUER (1943) for 8872 unhatched chicks. In these the proportion of males was only 44.7 percent. The contrast between that figure and all the others cited above (except the 40.1 percent for the writer's material) supports the other evidence that in this particular cross prenatal mortality of females is significantly higher than that of males. It is also higher than in females from other kinds of matings.

So far as the bare-back abnormality is concerned, the chicks that hatched late should probably be classified along with those that did not hatch, rather than with the ones that did. Ordinarily, the hatcheryman discards among the unhatched eggs a number with the shell pipped. In these two lots, some of the chicks managed to hatch after being brought to the laboratory, but among 39 such females the proportion with bare backs was 20.5 percent. This is much closer to the frequency of that abnormality among females of Classes 3 and 4 (table 6), in which it was 36.7 and 32.9 percent, respectively, than to its frequency of 0.9 percent among females that hatched on time. On this basis, if the data in table 6 are representative of other chicks from the cross under consideration, they show that for every bare-backed female that manages to hatch normally (in this case, 53) there are about five more that do not (in this case, 259). The loss to the hatcherymen is thus five times as great as they had supposed it to be.

The proportion of the non-barred chicks with extended black to which that genotype is lethal can be computed from the data for females in table 6. After deducting from the total of 7,329 such chicks the 476 with normal down

that failed to hatch, there remain 6,853 chicks. Among these there were altogether 312 classified as bare-backed, or 4.55 percent. This group, in turn, is divisible into two classes—the 61 that hatched and 251 that did not. The latter, which may be taken as the proportion of these females to which the extended black was lethal in this material, comprised 3.66 percent of the 6,853 black chicks.

B. *Evidence from sex-ratios in hatched chicks*

To determine whether or not extended black is lethal to a similar degree to black chicks of this same type from other flocks and other hatcheries, data were obtained from HALL BROTHERS HATCHERY, INC. of Wallingford, Conn., which now produces annually over 4,000,000 chicks from this cross. To get them it is necessary to incubate eggs from many different flocks and strains. For that reason, the chicks hatched should provide an adequate sample of all those from the cross of Barred Rock ♀ ♀ × Rhode Island Red ♂ ♂. If the short-down condition is lethal to a certain proportion of the chicks with extended black, there should be a deficiency of females among the chicks hatched, and that deficiency should be consistent over a period of years. The data in table 7, which cover more than 33 million chicks hatched in 10 years, were kindly provided by MR. STEPHEN WALFORD of Hall Brothers, and they show that both of these expectations were realized.

TABLE 7

Sex ratios and deficiencies of females among chicks hatched from the cross, Barred Rock ♀♀ × Rhode Island Red ♂♂.

Year	Males hatched		Deficiency of ♀♀	
	Number	Proportion of total	Number	Per cent
1938	517,998	50.8	- 16,957	3.3
1939	804,643	51.0	- 31,417	3.9
1940	806,751	51.0	- 30,902	3.8
1941	1,204,523	50.8	- 39,621	3.3
1942	1,684,023	51.1	- 72,240	4.4
1943	2,323,729	50.9	- 80,504	3.5
1944	1,796,461	50.8	- 58,846	3.3
1945	2,871,580	50.9	- 96,356	3.4
1946	2,318,160	50.9	- 85,948	3.7
1947	2,474,017	50.8	- 81,226	3.3
Totals and averages	16,801,885	50.9	- 594,027	3.54

In this table, the total number of chicks hatched each year and the numbers of females are omitted for simplification, but they can be easily computed from the data given. It will be noted that there was each year an excess of males over the 50 percent expected by chance, and a corresponding deficiency of females. Although the excess of males was small, it was consistent (varying only from 0.8 to 1.1 percent during the 10 years) and it was statistically

significant in each year. In the smallest population—the 1,019,039 chicks of 1938—the proportion of males was $50.8 \pm .049$ percent, but the deviation above 50 percent ($0.8 \pm .07$) is 11.3 times its standard error, and hence highly significant. Since in every other year the deviation was as great or greater, and the number of chicks was larger, the excess of males is significant in each case. That it resulted from a greater prenatal mortality of females was attested by LANDAUER (1943) who examined a large sample of chicks in eggs that had failed to hatch from this cross and from this same hatchery. In them, the females comprised 55.3 percent.

Because of the head spot which clearly differentiates males from females in this cross, and because the two sexes had to be thus separated before being sold, there can be no doubt of the accuracy of sex determination in these large numbers of chicks. There might still be some question, however, whether the consistent deficiency of females should be ascribed to some lethal effect of the extended black, or whether it resulted merely from causes similar to those that induce a deficiency of females in the secondary sex ratio of man, the dog, and some other mammals. This latter alternative seems unlikely for several reasons. In such mammals, the excess of males at birth apparently results from an even greater excess of males at fertilization. In contrast, such evidence as is available indicates that in the fowl the proportions of the two sexes are equal at fertilization (HAYS 1945). Moreover while the figures of various investigators for the proportion of males at hatching vary from 44.6 to 52.2 percent (for review, see HUTT 1949), most of the evidence from larger populations pointed toward there being fewer males than females until CREW (1938) found the proportion of males to be 50.3 percent in over half a million chicks hatched from various sex-linked crosses. The consistent excess of males shown in table 7 is in marked contrast with most of the previous records.

Finally, it is noteworthy that the annual deficiencies of females below the numbers of males vary only from 3.3 to 4.4 percent, and that the average deficiency of 3.54 percent was remarkably close to the lethality of 3.66 percent found by examination of the unhatched eggs from a different source. Considering all the evidence, it seems clear that in the cross of Barred Rock ♀ ♀ × Rhode Island ♂ ♂, the non-barred, extended-black condition is lethal to somewhat more than 3.5 percent of the resultant female chicks. In another fraction—about 0.8 to 1.0 percent—it shortens the down so much that the chick is recognizable at hatching as a "bare-back."

C. *Time of lethal action*

From the fact that the deficiency of females among the chicks hatched can be fully accounted for by the bare-backed ones found dead or unhatched after 15 days of development (table 6), it seems probable that the lethal effect of extended black is not exerted prior to the last week of incubation. In order to determine the lethal period more exactly, all the unhatched eggs obtained for examination from the local hatchery were classified according to the stage

of development of the chick. These examinations were made from 15 to 18 hours after completion of the full incubation period of 21 days.

At that time some of the unhatched chicks were still alive. Of these, some had been able not only to " pip " the shell, but also to rotate within it, breaking the shell as they went, thus leaving the large end " circled " as is normally done prior to hatching. Others had pipped the shell in one place, but were unable either to rotate or to circle the shell, or both. Some of these had managed to enlarge the hole, but all were classified as " holed." A few chicks were still alive but had not been able to pip the shell, and a few had even circled the upper end before dying. Among those that died without pipping the shell, it was possible to differentiate the ones that had succumbed after about 19 days of development from those that had died earlier by the position of the yolk-sac. This is normally drawn into the body on the nineteenth day, although occasionally in pathological cases it may still be outside of the body in chicks found alive but unhatched at 21 days.

The numbers of normal males, normal females and bare-backed females found in these various stages are given in table 8. Embryos that died prior to 16 days of development are excluded. Although one may not assume that every chick dead in a circled egg had lived longer than one in a holed egg, the stages listed in table 8 represent, in general, successively younger ages from top to bottom.

TABLE 8

Distributions at various stages of chicks that failed to hatch indicating the period at which bare females die.

Stage of development or of hatching	Normal males		Normal females		Bare females	
	Number	Per cent	Number	Per cent	Number	Per cent
Alive, pipped, circled	65	13.5	55	11.6	27	10.8
Alive, pipped, holed	55	11.4	50	10.5	44	17.5
Alive, unpipped	19	3.9	22	4.6	12	4.8
Dead, pipped, circled	16	3.3	16	3.4	5	2.0
Dead, pipped, holed	48	9.9	48	10.1	23	9.2
Dead, unpipped, yolks in	113	23.4	134	28.1	100	39.8
Dead, unpipped, yolks out	167	34.6	151	31.7	40	15.9
Totals	483	100.0	476	100.0	251	100.0

The males and females with normal down were distributed approximately alike among those various stages. With respect to the bare females, there were only 15.9 percent that died before inclusion of the yolk-sac, in contrast to the 34.6 and 31.7 percent of such chicks among the normal males and females. Evidently the lethal effect is exerted principally after the nineteenth day. The proportion of bare females that were alive in eggs that were pipped but not circled (17.5 percent) is somewhat higher than for normal chicks of both sexes. This suggests that some of these bare-backed females may die because of inability to rotate within the shell. However, the greatest excess of

bare females over the normal chicks was in those dead, unpipped, but with yolk-sac drawn in. Presumably these died at 19 to 21 days.

Altogether 44.3 percent of the bare chicks that failed to hatch were found alive or pipped, or both, and 39.8 percent more with yolk-sacs drawn in. It is clear, therefore, that the lethal effect is exerted in the final stages of incubation and in the preliminary stages of hatching. Since this happens also with the sex-linked mutation, naked, which is lethal to about half the naked chicks during the last three days of incubation (HUTT and STURKIE 1938), it seems possible that the extended black condition is not lethal *per se,* but that the cause of death is the extreme reduction of the down which it induces in some chicks. Considering both these partially lethal abnormalities, the possibility is suggested that the thick layer of down normally existing between the body of the chick and its embryonic membranes serves in some way to facilitate the process of hatching. If so, abnormality of that down must apparently be extensive before it can be lethal, for in chicks having the wiry, sticky, down of the mutation, " sunsuit," hatchability is normal (HUTT and LONG 1950).

PHYSIOLOGICAL EFFICIENCY OF BARE CHICKS

In 1946, 31 bare-backed females from this cross, and 25 normal ones from the same flock and the same hatch, were obtained for tests to determine whether or not the bare ones were in any way inferior to the others in viability after hatching, in growth, or in reproduction. These were reared together under normal conditions in brooders and on an open rearing range, were all housed together in one large laying pen at about 5 months of age, and were maintained under uniform environmental conditions until they were 380 days old. Comparisons of the two types by various measures of physiological efficiency are given in table 9.

TABLE 9

Various comparisons of normal females with those that were bare at hatching.

Measure of efficiency	Normal females	Females bare at hatching
Adult birds housed, number	22.	29.
Mean age at first egg, days	203.9 ± 4.3	204.4 ± 4.7
Mean body weight at first egg, grams	2551.1 ± 64.2	2560.0 ± 56.2
Mean eggs per bird to 380 days, number	140.0 ± 6.5	136.0 ± 8.7
Mortality to 380 days, per cent	24.0	32.3

The slightly higher mortality among females originally bare that is shown in table 9 is not significant. During the growing period, when the partially bare females might be thought to have some disadvantage, only 2 were lost, against 3 from their controls. Since the females originally bare proved to be not a whit inferior to their normal sisters in age at first egg, in body size, or in egg production, it seems clear that such chicks are normal in all respects and that their initial lack of down imposes no permanent ill effects. The fact

that the physiological efficiency is normal in these bare chicks that manage to hatch supports the other indications that the lethal effect just before hatching results from some impediment to hatching,—perhaps mechanical in nature —which is caused by the lack of down.

COUNTERACTION OF THE LETHAL EFFECT OF *E* BY THE GENE *k*

When it became clear that the gene *E* is sometimes lethal, experiments were undertaken to test the possibility of overcoming that influence. While the barring gene, *B*, does this nicely, as was shown earlier in this report, in the cross of barred ♀ ♀ × non-barred ♂ ♂, *B* can give its protection only to the male offspring and never to the non-barred females. It seemed possible that the recessive sex-linked gene *k* might serve the purpose because it induces more rapid growth of feathers after hatching than does its allele, *K*. The differing effects of these genes are most evident at 8 to 12 days after hatching, by which time the *k*– and *kk* chicks have little tails and wings almost as long as the body. At the same age, the *K*– and *KK* chicks have short wings and no tails. However, there is also a difference between these two types at hatching, when chicks with rapid feathering show markedly longer primary feathers in the wing than do those with slow feathering. It seemed possible that the same greater cellular activity in the feather follicles that accelerates the growth of these first teleoptiles in *k* chicks might also induce maximum development of the neossoptiles which comprise the down. Even if *k* does not cause elongation of these downy feathers, they might be forced farther out of their follicles by the developing teleoptiles that replace the down soon after hatching. Since both Barred Plymouth Rocks and Rhode Island Reds normally carry *K*, none of the offspring from their crossing have rapid feathering.

To test the possibility that *k* might counteract the effects of *E*, diallel crosses were arranged in which black females, some *K*– and others *k*–, were mated first with a black *kk* male and then some months later with one that was thought to be *KK*. Unfortunately this second male proved to be heterozygous (*Kk*), so not all of his progeny had slow feathering. The incidence of bare chicks in the black offspring of these crosses is shown in table 10.

These results indicate that the incidence of bare-backed chicks is greatest in the offspring of birds with slow feathering, and that the bare ones are reduced to the vanishing point when both parents have rapid feathering. However, the evidence is not as conclusive as the data in table 10 suggest. From Mating 4 (of table 10), among 78 chicks that lived to be classified at 8 and 10 days of age, there were 33 ♂ ♂ all either *KK* or *Kk*, 23 *K*– ♀ ♀, and 22 *k*– ♀ ♀, and in these three groups the proportions classified as bare were 30, 30, and 31 percent respectively. Since these numbers are small they are not too significant, but they do show that the gene *k* does not invariably overcome the tendency of *E* to reduce the down. The deficiency of 12 males in these same chicks suggests that a disproportionate number of that sex, all of which carried *K*, may have been among the 14 bare chicks that died during the late stages of incubation.

TABLE 10

Incidence of bare chicks in diallel crosses involving the K and k alleles.

Mating	Chicks* number	Proportion expected to carry K per cent	Bare* per cent	Dead-in-shell number	
				Not bare	bare
1. ♂ *kk* × ♀♀ *k*–	65	0	1.5	8	0
2. ♂ *kk* × ♀♀ *K*–	63	50	19.0	1	0
3. ♂ *Kk* × ♀♀ *k*–	79	50	11.4	10	1
4. ♂ *Kk* × ♀♀ *K*–	101	75	39.6	6	14

* Including dead-in-shell after 18 days of incubation.

Some difficulty was found in classifying these chicks, particularly those from Matings 1 and 2, which were hatched first. Some that were called bare at hatching showed little of that defect 24 hours later when they were thoroughly dry. It is probable, therefore, that some of these chicks from the *kk* sire described as bare would not have been so classified several months later when the chicks of Matings 3 and 4 were hatched. In these last the bareness was more pronounced, and of 40 then classified as bare, 13 were " extreme." None of this extreme kind was encountered in chicks from the *kk* sire. It is probable that they correspond to the type that the hatcherymen discard as too bare to sell.

It is significant that no chicks from the *kk* sire were found bare in the unhatched eggs. In contrast, in chicks from *Kk* ♂ × *K*– ♀, prenatal mortality was higher than in any of the other matings, and among the 20 that died unhatched no fewer than 14 showed bare-backs. Of these, 7 were classified as being extremely bare.

Altogether the evidence from these crosses indicates that *k* tends to prevent *E* from shortening the down to such an extent that it is conspicuously deficient at hatching, or lethal late in incubation. In practice, the poultry breeder could accomplish this desirable result (1) by using New Hampshire males, most of which are *kk*, for this cross, or (2) by substituting *k* for *K* in the Rhode Island Red, if he prefers that breed. In either case, the fact that the Barred Plymouth Rock females used are *K* would not matter, as their male offspring would be protected by the gene *B* received from their dams, and the female chicks by the gene *k* received from their sires. After these tests were completed, the writer was informed by DR. WALTER LANDAUER of the experiments of GREDINA (cited by LANDAUER 1948) which indicated that even in pure Rhode Island Reds, the gene *k* is more conducive than *K* to hatchability. In her three populations of chicks, (1) all with rapid feathering, (2) half rapid and half slow, and (3) all slow, the hatchability of fertile eggs was respectively 61, 54, and 45 percent. Tests of the relationship with larger numbers and under environmental conditions that permit higher levels of hatchability are desirable, but her findings agree with the writer's in suggesting that *K* may be detrimental, and *k* advantageous, in the complicated process of transforming an egg into a chick.

DISCUSSION

There are in animals a number of well-known cases in which a certain color or pattern is associated with some physiological deficiency, or with lack of viability, but in none of these, so far as the writer is aware, does that effect result from a reduction of the hair or plumage, as in the present case. Similarly, while black mice are smaller than brown ones (GREEN 1931; LAW 1938), and black rats are more placid than grey ones (KEELER 1942) there seems to be no record in these well-studied species, or in others, of an association between color of the hair and its length.

It has been found by several investigators that in black feathers the pigment granules differ in size and shape from those of red and buff feathers. HAMILTON (1940) has shown that melanophores have lower viability in white fowls than in black ones. The mechanism by which black pigmentation shortens the down has yet to be discovered. Hatcherymen have reported that the incidence of bare-backed chicks varies in eggs from different flocks, even though in all cases they come from Barred Rock females mated with Rhode Island Red males. Such differences may result from the fact that some breeders of Rhode Island Reds are now selecting for rapid feathering and have already substituted *k* for *K* in much of their stock. The conditions during which eggs are held prior to incubation and the duration of that holding period are also said to influence the frequency of the defect.

The possibility that the reduction of the down and associated mortality of black chicks during incubation is not induced by *E*, but, rather, by some other gene linked with *E*, would seem to be eliminated by two facts. In the first place, no cross-overs (*i.e.*, no bare chicks) occurred among 219 chicks with restricted black (*ee*) that segregated from *Ee* dams (table 1). Secondly, the fact that dilution of the black by the barring gene lengthens the down (tables 2 and 4) and practically eliminates the bare-back condition (table 6) indicates that the trouble is primarily associated with the extreme melanosis that is induced by *E*. It is to be expected, therefore, that the same abnormalities would be found in black, slowly-feathering chicks of breeds or crosses other than that concerned in the present investigation. (After this manuscript had been submitted, the author found these same bare-backed black chicks in England in totally unrelated stock from the cross Rhode Island Red ♂ × Black Leghorn ♀.)

The fact that the black, bare-backed chicks which die late in incubation have knotty, clubbed down like that frequently seen in embryos that die from a deficiency of riboflavin suggests that the black ones may need abnormally high amounts of that vitamin. Genetic differences among fowls in requirement of riboflavin do exist (LAMOREUX and HUTT 1948) but any possible relationship of such differences to the gene for extension of black remains to be tested.

As statistics are not available on the numbers of chicks hatched annually from this cross, the author has made no attempt to estimate the economic loss caused by the gene *E*. Geneticists interested in this point should inspect

the data from a single large hatchery given in table 8. In one decade, this hatchery lost 594,027 female chicks worth about 30 cents each, or a total of $178,208. That figure does not include the additional loss from chicks that did hatch but were too bare to sell. If further evidence should confirm the indications already found that such losses can be avoided or reduced by substituting *k* for *K* in the non-barred males used in this cross, a useful application of genetics in agricultural practice will have been demonstrated.

SUMMARY

Among chicks from the cross, Barred Plymouth Rock ♀ ♀ × Rhode Island Red ♂ ♂, about one percent or more of the females show bare backs at hatching. These chicks lack much of the down in the posterior-spinal feather tract, and what remains is abnormally short. There are all gradations between extreme variants and the normal. Males are affected so rarely that none with bare back may be seen among many thousands.

Genetic analysis showed that the defect is found only in chicks with extended black (*E*), and never in their siblings with Columbian restriction (*ee*). This association results from a pleiotropic effect of *E*, which shortens the plumules that comprise the down. The shortening is not localized on the back, although it is there most conspicuous.

Measurements of feathers showed that the sex-linked gene for barring (*B*) prevents *E* from shortening the down excessively and thus protects the male chicks from this cross. The silver and gold alleles (*S* and *s*) do not affect the length of the down.

Reduction of the down induced by *E* is lethal to about 3.5 to 3.7 percent of the black, non-barred females from this cross. This was shown not only by significant deficiencies of females in over 33 million chicks hatched from this cross, but also by a corresponding excess of females with bare backs found among an adequate sample of eggs that failed to hatch. The deaths occur chiefly during the last two days of incubation. Since the female chicks are much more valuable than the males, the loss is doubly serious. It was found that this lethal effect of *E* cost one hatchery alone over $178,000 in a decade.

Bare-backed females that did hatch were fully equal to their normal sisters in viability, age at first egg, body weight and egg production.

Evidence was found by diallel crosses that the chicks with extremely bare backs may be avoided, and the lethal action of *E* as well, by using sires that have rapid feathering (*kk*) rather than slow feathering (*Kk* or *KK*).

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EVIDENCE FOR A SEX-LINKED LETHAL IN THE HOUSE MOUSE[1]

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EVIDENCE of sex-linked inheritance in rodents has so far been limited to a few incompletely documented instances encountered mainly in the mouse. While the cytological prerequisite for X-linked loci seems provided by a differential non-pairing segment of the X-chromosome in some species such as the Norway rat (KOLLER and DARLINGTON 1934), the same cannot be said with certainty for the XY pachytene complex of the mouse as pictured by SLIZYNSKI (1949).

Observations by CHASE (1946) of polydactylism with about 25 percent manifestation constitute probably the first clear-cut case of sex-linkage in the mouse, aside from the earlier more indirect demonstration of a sex-linked tumor-transplantability factor by STRONG (1929) and by BITTNER (1932). WRIGHT (1947) has shown recombination between "shaker" and sex, and between "wavy" and sex; however, the recombination fractions between these two genes and sex are both in excess of 50 percent, and are almost identical in spite of a map-distance of about 35 crossover units between wavy and shaker. In an effort to interpret WRIGHT's admittedly paradoxical data, FISHER, LYON and OWEN (1947) have proposed a mathematical formulation of sex-linkage relationships for which cytological support is still to be sought.

Possible sex-linked lethals have been reported by MORGAN (1914), and by LITTLE (1920) in a line of Japanese waltzing mice. SNELL (1931) regards the proof in these two instances as inconclusive, for "in neither case was it shown that the lethal was transmitted to half the daughters of an affected female, the other half being normal, as should be the case with a sex-linked lethal, and in other respects the data are rather incomplete."

The problem of what constitutes normal sex ratio in the mouse is complicated by controversy regarding environmental influences. Sex ratio has been supposed to be modifiable in either the male or the female direction by season (PARKES 1924); alcohol (BLUHM 1921, 1930 and numerous other papers; repeated by CHAUDHURI 1928; but convincingly refuted by CREW 1926, and by MACDOWELL and LORD 1927 a, b); sodium bicarbonate (BLUHM 1932); polygyny (PARKES 1925; not confirmed by GATES 1930); and diet (BITTNER 1936 a, b).

[1] Aided by an institutional grant from the AMERICAN CANCER SOCIETY.

The present experiments were aimed at clarifying the genetic background of a lethal sex ratio, first encountered four years ago in an isogenic brother-sister subline of Strong A-mice. This subline was maintained by selection under adequate control for those extrinsic factors which past work has implicated as capable of modifying sex ratio. Our data represent observations on over 4,000 A-mice (exclusive of controls and outcrosses) reared in this laboratory since 1946 over eleven successive generations.

MATERIALS AND PROCEDURE

The parental generation of our A-strain mice was received in September 1946 from DR. W. E. HESTON, whose colony at the NATIONAL CANCER INSTITUTE stems from A-mice obtained from the ROSCOE B. JACKSON MEMORIAL LABORATORY in 1938. HESTON and DERINGER (1947) consider the strain "homozygous for practically all genetic factors." Brother-sister inbred generations now total over 90. Sex ratio at birth is normal both in DR. HESTON's A-mice (HESTON 1948) and in the original stock carried by DR. L. C. STRONG through F_{92} (STRONG 1950b). Sublines were established by us through selection on the basis of low (2 ♀ ♀ : 1 ♂) or normal (1 ♀ : 1 ♂) sex-ratio. All matings were brother to sister, except for exploratory outcrosses to C3H-strain followed by backcrosses to A.

The animals were kept in stainless steel cages measuring $12 \times 6 \times 6$ inches. Usually two or three females were mated to one brother. All breeders bore ear numbers. Pregnant females were isolated in individual cages, the young were sexed at birth with the aid of a dissecting microscope and sexed again at weaning age of 3 to 4 weeks, whereupon the mothers were immediately remated. The number of litters per female in most cases was not allowed to exceed four, because litter size drops off sharply with increasing maternal age. A representative series of 210 first through sixth A-strain litters averaged 4.63, 6.27, 6.20, 5.87, 5.24, 3.44 young at birth.

More than the usual hygienic precautions were observed since disease had to be ruled out as a possible contributory factor to differential male mortality. Cages, food-hampers and water bottles were cleaned and autoclaved twice a week. Sterilized wood shavings served for bedding. A mercury thermocouple, wired to an auxiliary heater in the animal room, was set to prevent a temperature drop below $70°F \pm 2°$ at any time. By these protective measures a dependable state of good health was achieved.

The balanced Morris-Thompson checker diet (MORRIS 1944) was fed ad libitum throughout the 42-month period under consideration. In one control experiment this feed was supplemented with whole oats and peanuts, since elusive dietary effects on sex ratio had been encountered by BITTNER (1936 a, b).

The data were collected in such a way that information was available not only on lineage and sex ratio at birth, but also on intervals between mating and birth, sex of abnormalities such as "harelip" and "eyelid open" observed at birth, post-natal depletion, and sex ratio at weaning. This continuous record now covers 11 successive generations.

In the statistical treatment the standard error has been used in preference to the probable error.

For preliminary clues as to the developmental stage at which the supposed lethal takes effect, the ovaries and uteri of 51 mice with known normal or abnormal sex ratio trends were dissected in advanced stages of gestation. The corpora lutea of pregnancy, placental resorption sites and viable embryos were counted; pre-and post-implantation losses were thus numerically distinguishable.

RESULTS

1. Sex ratio

In December 1946, it was noticed that some of the daughters of A-strain ♀ No. 7 (one of the mice received three months earlier from DR. HESTON) produced litters consistently deficient in males. The over-all sex ratio at birth was 43.7 percent or about four females per three males, as might result from the combined output of two categories of F_1 breeders producing at the rates of 1:1 and 2:1, respectively. This initiated the establishment of a subline wherein systematic selection for low sex ratio was to be carried out through several generations.

The male-deficient subline (designated as A7 after the ear punch number of its maternal ancestor) did not breed true, for it contained both normal and low sex ratio females in each generation. A true-breeding control subline A7N stemmed from several normal A7 females which had been tested for

TABLE 1

Comparison of two typical maternal sex ratio lineages in sublines A7 and A3 of the A-strain mouse.

Generation	*Line A7** (2:1 sex ratio)		*Line A3* (1:1 sex ratio)	
		♀♀ ♂♂		♀♀ ♂♂
F_9	♀ No. 1448	10: 5	♀ No. 1603	3: 4
F_8	♀ No. 1405	19:13	♀ No. 1536	3: 3
F_7	♀ No. 1357	7: 4	♀ No. 1463	9: 7
F_6	♀ No. 1327	23:13	♀ No. 1403	7: 6
F_5	♀ No. 1220	22:13	♀ No. 1092	16:15
F_4	♀ No. 990	9: 4	♀ No. 905	6: 3
F_3	♀ No. 364	8: 3	♀ No. 710	4:10
F_2	♀ No. 210	6: 1	♀ No. 104	5: 8
F_1	♀ No. 51	14: 7	♀ No. 4	8: 6
P	♀ No. 7	14:10	♀ No. 3	3: 5
Totals		132:73		64:67
	Sex ratio = 35.12% ♂♂		Sex ratio = 50.81% ♂♂	

*** The 9 females of subline A7 (generations F_1–F_9) traced through their maternal descent in table 1 had altogether 23 sisters; 15 of these gave normal sex ratio of 87 ♀♀:105 ♂♂ (s.r. = 54.68%); 5 gave "lethal" sex ratio, totalling 58 ♀♀:25 ♂♂ (s.r. = 30.12%); 1 was sterile, and for 2 the litters could not be sexed at birth because of cannibalism. Within this representative random sample of subline A7 we find, therefore, a total of 15 females reproducing at an average of 1 daughter:1 son, 14 females averaging 2 daughters:1 son, and 3 unclassified breeders.**

TABLE 2

Sex ratios for sublines of the A-strain mouse and other strains bred under same dietary and other environmental conditions (based on 16,091 mice).

Strain	Type of mating	Selection for sex ratio	Time sexed	♀♀	♂♂	Total	Sex ratio (% ♂♂)
A Line 7 ($P-F_4$)	b.s.	low S.R.	birth	944	753	1697	44.37 ± 1.20
A Line 7 (F_7-F_8)	b.s.	S.R. allowed to drift	birth	833	751	1584	47.41 ± 1.22
A Line 7N	b.s.	derived from line 7 ♀♀ with normal S.R.	birth	301	302	603	50.08 ± 2.03
A Line 3	b.s.	none	weaning	127	126	253	49.80 ± 3.13
Strong A*	b.s.	none	birth	109	127	236	53.82 ± 3.25
A × C3H (Heston)	o. and b.	A-parents from low S.R. lineage	birth	412	447	859	52.04 ± 1.70
Heston C3H + milk factor	b.s.	none	weaning	329	337	666	50.60 ± 1.94
Strong C3H + milk factor	b.s.	none	birth	209	223	432	51.62 ± 2.41
Andervont C3H − milk factor	b.s.	none	birth	213	226	439	51.48 ± 2.39
Little dba	b.s.	none	weaning	409	422	831	50.78 ± 1.73
Little dba	b.s.	none	birth	199	220	419	52.50 ± 2.43
MacDowell C58	b.s.	none	birth	44	71	115	61.74 ± 4.66
C57 black	b.s.	none	birth	378	450	828	54.35 ± 1.74
CAF (A × Bagg Albino C)	random	none	weaning	1200	1257	2457	51.11 ± 1.00
Webster Swiss	random	none	birth	2279	2393	4672	51.22 ± 0.73

Symbols used in table 2: b.s. = brother-sister; S.R. = sex ratio; o. and b. = outcrosses and back-crosses.

*Derived from two pairs received directly from Dr. L. C. Strong for comparison with sex ratios in our A-strain sublines.

consistent 1 : 1 ratio. An additional control line A3 was derived from A-stock prior to the A7 branch. The patterns of A7 and A3 with regard to sex ratios are evident from table 1 which shows two typical ancestral tracings for two F_9 females picked at random.

Sex ratios, aims of selection and types of mating for A7, A7N, outcrosses and other strains simultaneously reared in this laboratory under comparable environmental conditions are summarized in table 2. Four conclusions may be drawn from tables 1 and 2:

1. Most strains of mice reproduce at normal sex ratio.
2. Low sex ratio is not an inherent feature of the A-strain as a whole, but its genetic control is such that it may be held near the 4 ♀♀ : 3 ♂♂ level by continuous selection, or at the 1 ♀ : 1 ♂ level without further selection.
3. Sex ratio is apparently not raised much above 50 percent as a result of heterosis, the ratios for the random-bred heterozygous CAF and Swiss mice being comparable with those for the isogenic A7N, A3, Strong A, C3H, dba, and C57 black strains. (The unusually high sex ratio in the C58 strain is based on too small a sample to permit speculation.)
4. Environmental influences could not have been responsible for the divergent trends in ratio, since the different sublines and strains were reared simultaneously under identical conditions.

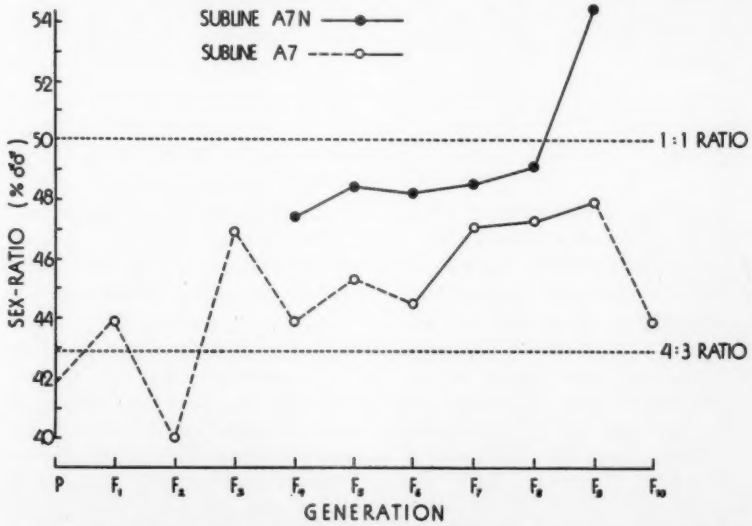

FIGURE 1.—Sex ratio trends in sublines A7 and A7N of the A-strain mouse. Broken line covers periods of selection for low sex ratio. Solid line for generations F_6–F_9 of subline A7 shows interval during which sex ratio was allowed to drift without selection. A7N was derived from A7 mothers with demonstrated normal sex-ratio.

In subline A7, breeders were selected only from litters in which daughters outnumbered sons on an average of 2:1 and which had been preceded by from one to three litters with a similar excess of daughters.

On the assumption of a sex-linked lethal factor inherited by only one half of the females in each generation, this mating scheme should ideally result in a population sex ratio of 43 percent, with which our experimental result of 44.37 ± 1.20 agrees rather well. When, after six generations, systematic selection was temporarily abandoned there was an immediate upswing toward normalcy, followed by a rapid return to 44 percent in consequence of resumed selective mating. Figure 1 illustrates this point graphically. The rapid shifts brought about by discontinuous selection in A7, and occurring either upward or downward within one generation, make it apparent that we are here not dealing with the gradual trends typical for multiple factor control but with few genes, possibly with a single pair of alleles. The suggestive upward trend of the control ratios in A7N (which was extracted from normal A7 females) could mean that minor modifiers for male deficiency—in addition to the principal gene(s)—had been concentrated in the stock and were gradually reshuffled after selection ceased.

II. Bimodality of sex ratio distribution

Among the important criteria for sex-linkage of a lethal is the proof that it is transmitted through only one of the two X chromosomes of a "carrier"; the "carriers" in the present case being females of the hypothetical constitution X^+X^1 whose offspring would segregate into four numerically equal genotypes:

1. Normal ♀ ♀ X^+X^+ reproducing at a 1:1 sex ratio
2. Carrier ♀ ♀ X^+X^1 reproducing at a 2:1 sex ratio
3. Normal ♂ ♂ X^+Y producing X and Y spermatozoa
4. Non-viable ♂ ♂ X^1Y which usually die before birth

The balanced bimodality of sex ratio distribution (fig. 2) for generations F_1–F_6 demonstrates that subline A7 is indeed composed of two distinct classes of females, while the normal subline A7N comprises only one type.

The combined output of all the A7 breeders (96 ♀ ♀ of generations F_1–F_6) represented in figure 2 was 944 ♀ ♀ : 753 ♂ ♂, or 44.37 ± 1.20 percent ♂ ♂. Expectation on the basis of the ideal 4:3 or 42.9 percent ratio is 969 ♀ ♀ : 728 ♂ ♂. Chi square for one degree of freedom = 1.290; P = 0.2–0.3. Hence the A7 data are in good agreement with the theoretical population ratio under the "lethal" hypothesis. On the other hand, these results depart significantly from the expectation of a normal sex ratio. Chi square for deviation from 50 percent = 21.736; P = < 0.0001. Conversely, the output of 45 breeders of subline A7N shown in figure 2 was 301 ♀ ♀ : 302 ♂ ♂, or 50.08 ± 2.03 percent ♂ ♂. Chi square for one degree of freedom = 0.014; P = 0.90.

A further analysis of the data was made by considering the distribution of the sexes in the progeny of all A7 females (71 ♀ ♀ of generations F_1–F_6)

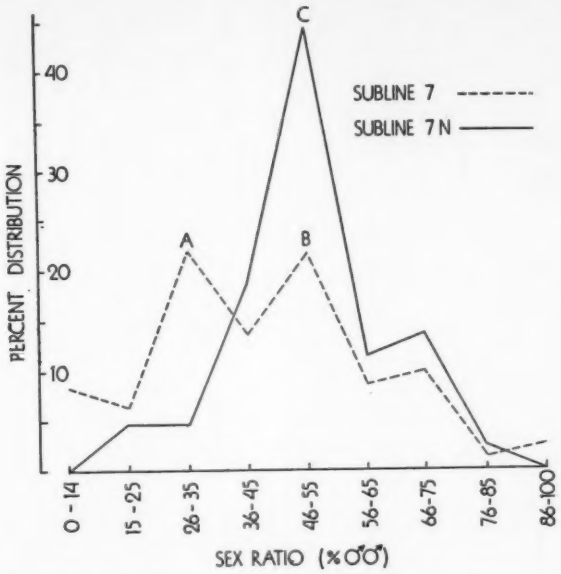

FIGURE 2.—Bimodal distribution of sex ratio in litters of subline A7 (generations F_1–F_8) and unimodal behavior of control subline A7N. The two modes for A7 are at 33% and 50%. The single peak for A7N lies at 50%. (Based on a total of 1697 A7 and 603 A7N mice.)

which had totalled ten or more young. In figure 3, the number of sons is plotted against the number of daughters produced by each mother. Distribution of the points shows bimodal clustering along two axes, one at a sex ratio of 50 percent, the other at 33 percent.

Statistically, each breeding female in figure 3 was regarded as an entity and the squares of deviations of individual output from expectation were taken into account (table 3). Thus, the 37 females in Class I allowed 36 degrees of freedom. More convincing than the obviously significant difference between Class II and the normal Class I (D/SE = 6.5) is the close fit of Class II performance for the lethal 33.3 percent sex ratio. Chi square for 33 degrees of freedom = 34.33; $P = > 0.3$.

III. Outcrosses and backcrosses

The A7 males apparently did not transmit the principal factor(s) responsible for the abnormal sex ratio, since within numerous mating groups of 2 to 5 sisters by a single brother the sisters clearly fitted into both the 1 : 1 and 2 : 1 reproductive categories.

When A7 males from litters with a 33 percent sex ratio were outcrossed to C3H females from a line with a mean sex ratio of 50.60 ± 1.94, the progeny

TABLE 3

Analysis of A7 females which had born a sufficiently large number of young to permit their probable individual classification as genotype $X^+ X^+$ (I) or genotype $X^+ X^1$ (II).

Class	No. of breeding ♀♀	Mean No. of litters per ♀	Mean No. of young per ♀	Total daughters : sons	Mean sex ratio	Degrees of freedom	Chi square for dev. from 50%	P
I								
S.R. over 41%	37	3.41	17.9	325:336	50.83% ± 1.94	36	36.74	> 0.3
II								
S.R. under 41%	34	3.38	19.8	447:226	33.58% ± 1.79	33	230.34	< 0.0001

TABLE 5

Corpora lutea of pregnancy, loss of ova, and death of embryos in 51 mice of sublines A7N and A7.

History of pregnant females	Total young in previous litters ♀:♂	Sex ratio at birth	Living embryos per current litter	Corpora lutea per mouse	Ova lost prior to implant. per mouse	Primary litter size (= number implanted)	Placental resorptions per mouse	Dead embryos (as percent of primary litter size)
Group A 14 mice with normal sex ratio whose mothers showed a 1 : 1 ratio	75: 74	49.6	7.0	9.08	1.16 ± 0.63	7.92	0.92 ± 0.34	11.6
Group B 26 mice with normal sex ratio whose mothers showed a 2 : 1 ratio	133:181	57.6	7.9	10.04	0.88 ± 0.20	9.16	1.23 ± 0.19	13.4
Group C 11 mice with 2 : 1 sex ratio whose mothers showed a 2 : 1 ratio	145: 74	33.8	7.0	10.64	1.36 ± 0.48	9.28	2.27 ± 0.27	24.5

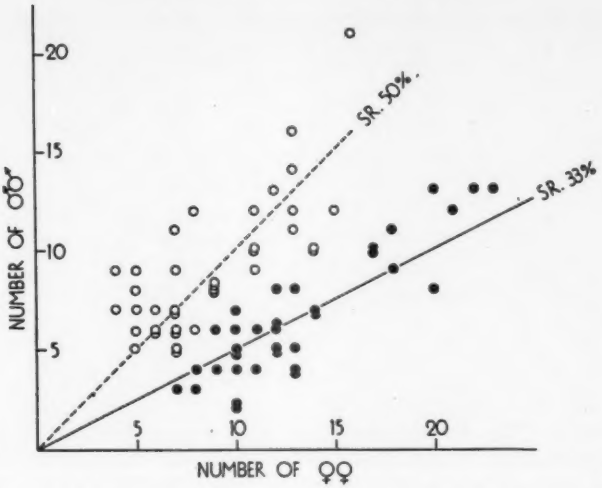

FIGURE 3.—Individual sex ratio performance of all A7 mothers (generations F_1–F_6) with 10 or more offspring. Open circles represent breeders giving sex ratios above 41%. Black circles stand for sex ratios of 41% or less. (Based on 71 mothers with a total of 1334 young.)

gave 50.1 percent (table 4). The results for backcrosses of the F_1 hybrid daughters to their A7 fathers and of the backcross-daughters to their A7 grandfathers were likewise normal.

Contrary to expectation, the reciprocal outcross of A7 females by C3H males also gave a 1 : 1 ratio, instead of 4 : 3 (table 4). The complete absence of "penetrance" of the sex ratio depressant(s) in the heterozygous F_1 animals could mean that recessive plus-modifiers favoring expression of the lethal in the homozygous A7 genotype were neutralized by dominant alleles introduced from C3H.

IV. Anatomical evidence

Although the breeding statistics for the 33.3 percent sex ratio of about half of the A7 females are convincing, it is conceivable that this anomaly

TABLE 4

Sex ratio in outcrosses and backcrosses.

Cross	Number of litters	Mean litter size	Total young ♀♀ : ♂♂	Sex ratio (% ♂♂)
13 A ♂♂ × C3H ♀♀	13	7.0	45 : 46	50.1 ± 5.2
26 F_1 ♀♀ × A-fathers	30	6.3	83 : 107	56.3 ± 3.6
24 F_2 ♀♀ × A-grandfathers	40	4.8	102 : 91	47.2 ± 3.6
33 A ♀♀ × C3H ♂♂	54	7.1	182 : 203	52.7 ± 2.5

might result through some obscure genetic mechanism other than death of one half of the embryonic males.

Data for embryonic death and resorption were, therefore, obtained from dissections of 51 pregnant A7N and A7 females (table 5). These mice were of comparable age and all had previously produced litters of known sex ratio. They were sacrificed between about the 12th and 18th days of gestation at which time the enlarged orange-colored corpora lutea of the current pregnancy were easily distinguishable from the degenerating previous corpora and could be accurately counted. The corpora lutea of pregnancy equal the number of eggs ovulated during the last estrus. Comparison of this count with the number of placental resorption sites and viable embryos (figs. 4, 5) gave exact quantitative information regarding pre- and post-implantation losses.

The three groups of pregnancies in table 5 are classified according to the previously established individual sex ratios of the dissected females and of their mothers. Thus, group A consists of 14 mice from subline A7N which were the daughters of A7 mice with demonstrated normal sex ratio. Group B comprises 26 normal daughters, and group C 11 " carrier " daughters of A7 mice with lethal sex ratio, whose maternal ancestors over at least four consecutive generations had been selected for low sex ratio (compare table 1).

The means for corpora lutea of pregnancy were of the same order of magnitude in normal and " carrier " mice. Group C shows a somewhat higher pre-implantation loss than A and B; however, the large standard errors in this category are indicative of considerable variability and the differences are not significant.

Placental resorption sites, on the other hand, were about twice as frequent in the uteri of the " carrier " group C than in the two control groups. Resorption frequency ranged from 0–4 per female. Comparison of the three groups on a percentage basis shows fairly consistent trends (table 6).

The differences between the resorption means of groups A and C, and B and C (table 5) are 1.35 ± 0.43 and 1.04 ± 0.33. Both differences are more than three times their standard error, hence significant. This indicates that the lethal exerts its effect after implantation.

TABLE 6

Frequency of placental resorption in 3 groups of pregnant A-strain mice with known sex ratio background (based on same groups as table 5).

Number of placental resorptions per pregnancy	Percent distribution		
	A 1:1 daughters of 1:1 mothers	B 1:1 daughters of 2:1 mothers	C 2:1 daughters of 2:1 mothers
0	58	19	0
1	21	50	19
2	15	19	36
3	0	12	45
4	6	0	0

FIGURE 4.—Dissection of pregnant uterus of an A7 carrier female which had given birth to 21 daughters and 11 sons (2:1 ratio). Corpora lutea of the current pregnancy numbered 10; 5 embryos were fully formed and 3 placental resorption sites—indicated by arrows—were found. Pre-implantation loss in this case amounted to 2 ova.

FIGURE 5.—Pregnant uterus of an A7N female with normal sex ratio. No placental resorptions occurred in this individual, despite the relatively large litter size.

TABLE 7

Correlation between litter size and sex ratio at birth in subline A7 of the A-strain mouse.

Sex ratio (% ♂♂)	Total litters	Total young	Mean litter size	Percent distribution of litter sizes 1–12 at birth											
				1	2	3	4	5	6	7	8	9	10	11	12
I															
All litters with S.R. 50% or above; Combined S.R. = 63.9%	285	1419	4.98 ± 0.12	1.7	10.9	9.9	21.7	16.5	18.2	8.7	7.7	4.2	0.5	....	....
II															
All litters with S.R. below 50%; Combined S.R. = 27.9%	252	1420	5.63 ± 0.13	1.9	4.4	10.7	12.3	16.6	21.5	13.1	10.7	5.6	2.8	....	0.4

TABLE 8

Mortality of young between birth and weaning, based on 1106 mice born by A7N and A7 ♀♀ with established 1:1 and 2:1 sex ratios.

	Class I Offspring of mothers with established 1:1 sex ratio			Class II Offspring of mothers with established 2:1 sex ratio		
	♀♀	♂♂	Sex ratio	♀♀	♂♂	Sex ratio
Total sexed at birth	317	342	51.9	300	147	32.9
Immediate loss due to harelip, monstrosities, still-births, cannibalism	37 = 11.7%	55 = 16.1%	59.8	31 = 10.3%	19 = 12.9%	38.0
Depletion of litters between 2nd and 21st day after birth (causes undetermined)	22 = 6.9%	15 = 4.4%	40.6	19 = 6.4%	12 = 8.2%	38.7
Total weaned at 21 days	258	272	51.3	250	116	31.7
Total loss and sex ratio of loss	59 = 18.6%	70 = 20.5%	54.3	50 = 16.7%	31 = 21.1%	38.3

Typical dissections are pictured in figures 4 and 5. The observed resorption sites and dead embryos ranged from the early limbbud stage to about the 12th day of gestation. Death of the missing males, therefore, does not seem to occur at a rigidly fixed developmental level. Histological determination of the sex of resorbing embryos was not feasible, since gonadal differentiation in the mouse does not begin until the 12th day post coitum (BRAMBELL 1927 a, b).

If the non-viable embryos in group C of table 5 were all males (as deducible from the 2:1 sex ratio at birth), then the pre-natal mortality rate of 24.5 percent based on primary litter size would account for the death of 49.0 percent of the male embryos, *i.e.*, half the sons of " carrier " females. Since the sex ratios for the control groups A and B were normal, the two means of 11.6 percent and 13.4 percent embryonic death apply equally to sons and daughters. The group C data might accordingly be corrected downward to from 35.6 percent to 37.4 percent of male embryos lost through the sex-linked lethal mechanism. These values differ significantly from the controls and lie well within the limits of chance variability around the expected 50 percent in view of the moderate number of females dissected. The anatomical observations alone do not, of course, constitute proof for sex-linkage of the lethal; but they are consistent with the statistical evidence for the 2:1 sex ratio and for the bimodality of sex ratio distribution.

V. Litter size

One would anticipate the litters of normal A7 females to be 25 percent larger than those of their " carrier " sisters, the litters of the latter being depleted by prenatal death of about half the male embryos. This statistical expectation was not borne out by the results summarized in table 7.

Litter size frequency followed a bimodal distribution with one mode at 4 young, typical for normal mice, and another at 6, representative of mice with low and lethal sex ratio. A distribution shift in the opposite direction would have been more in keeping with the sex-linked lethal hypothesis. One possible explanation for this paradoxical " compensating " phenomenon will be discussed below.

The number of litters (537) and young (2839) included in table 7 is large enough and the trends in the two categories of breeding females are consistent enough to impart significance to the difference in litter size between females of classes I (4.98 ± 0.12) and II (5.63 ± 0.13). Mean litter size of the latter exceeds that of the former by 0.65 ± 0.176, or 3.61 times the standard error.

VI. Absence of dietary effects

Since BITTNER (1936 a, b) has observed dietary deficiency effects on sex ratio, the Morris-Thompson checker diet (MORRIS 1944) was supplemented with whole oats and peanuts in a group of 19 A7 females mated to their brothers. These mice produced 32 litters sexed at birth as 97 ♀♀ : 74 ♂♂, or 43.3 ± 3.8 percent, which approximates the data for subline A7 as a whole.

The enriched diet did not alter the typical 4:3 sex ratio of line A7 at birth or at weaning. Furthermore, A7N mice and other strains receiving only the checker feed for the past three to four years have continued to reproduce at normal ratios and with unimpaired fertility.

Diet might conceivably affect survival of young through its influence on lactation, bulk of intestinal contents, or endemic diseases, such as infantile diarrhea. The latter has been found by HESTON (personal communication) to kill more nursing males than females, thereby lowering sex ratio in the A-strain mouse from normal at birth to 42.05 ± 1.59 percent at weaning time.

VII. Post-natal mortality

While the low sex ratios at birth in our subline A7 do not, of course, result from either diet or disease, it was of interest to determine whether environmental or genetic factors produced a further differential loss of males between birth and weaning. Post-natal mortality (table 8) in the progeny of two classes of A-females whose 1:1 or 2:1 sex ratio trend had been established by previous litters, was of the same order of magnitude.

Death due to harelip (*hp*) and consequent inability to nurse accounted for over 80 percent of the early post-natal depletion of litters and eliminated slightly more males than females. The relatively high incidence of the gene *hp* was, however, in no way associated with the lethal sex ratio in the A7 stock, as cleft palate occurred with similar frequency in the normal control sublines A7N and A3.

Another abnormality observed in newborn A7 mice was "eyelid open at birth" (suggested symbol *o*). This recessive manifested itself in 59 ♀ ♀ and 127 ♂ ♂ from 150 litters totalling 1014 young, *i.e.*, about one-fourth of the litters sexed at birth. Expression in males was twice as frequent as in females which agrees with LOEFFLER's (1932) finding for a similar character in mice derived from Agnes Bluhm's stock. Unilateral dextrous *o* was 1.45 times more frequent than sinistrous manifestation. Bilateral *o* was seen in only 8 out of the 186 affected mice. Animals exhibiting *o* were of somewhat reduced viability; but there was no indication that this gene and its modifiers partook in the mechanism responsible for the lethal sex ratio.

VIII. Mother's parturition age

The possibility that sex ratio may shift with increasing maternal age is suggested by KING's (1927) results for rats which gave birth to a significant excess of daughters toward the end of their reproductive life. Most of our A7 mice were not allowed to produce more than three litters, *i.e.*, they were retired when about six months old. Within this category of mothers, age showed no relation to sex ratio, the separate means for first, second and third litters all approaching the expected 43 percent.

A definite influence of mother's parturition age on sex ratio—but in the direction of normalcy rather than female excess (cf. KING 1927)—became apparent from the analysis of those A7 breeders, 46 in all, which had borne four or more litters (table 9).

TABLE 9

Parturition age of mother and sex ratio (based on all 46 A7 females which had produced 4 or more litters)

Litter order	Number of litters*	Mean litter size	Total ♀♀	Total ♂♂	Sex ratio %
1	38	4.63	123	53	30.12 ± 3.45
2	41	6.27	158	99	38.52 ± 3.04
3	44	6.20	164	105	39.03 ± 2.98
4	46	5.87	139	131	48.52 ± 3.20
5+	41	4.53	101	85	45.69 ± 3.62

*Some litters were eaten or mutilated by their mothers before they could be sexed. Only intact litters sexed at birth are included.

All but eight of these females had a significant excess of daughters in each of their first three litters, hence most of them were probably heterozygous for the lethal. The combined progeny and sex ratio at birth for the first three litters (table 9, lines 1–3) was 445 ♀ ♀ : 257 ♂ ♂ = 36.61 ± 1.84 percent, while the fourth, fifth and subsequent litters were composed of 240 ♀ ♀ : 216 ♂ ♂ = 47.37 ± 2.34 percent. The difference between the sex ratios of the first three and later litters is 10.76 ± 2.98, or 3.6 times its standard error, hence significant.

DISCUSSION

Some of the ill-documented invocations of sex-linkage in the literature evaluated by GRÜNEBERG (1943) justify the cautioning statement that "the introduction *ad hoc* of sex-linked genes may be in formal agreement with the observed facts and yet the postulated sex-linked genes may not exist." With full appreciation of this possibility we are here on somewhat safer ground than the work referred to by GRÜNEBERG, since we are not concerned with unequal distribution of a morphologic trait between the two sexes, but with sex ratio proper.

Neither diet nor other environmental factors appeared to modify the proportion of the sexes at birth under our experimental conditions. The significant shift from 2 ♀ ♀ : 1 ♂ toward 1 ♀ : 1 ♂, found to coincide with increasing parturition age in "carrier" females of subline A7 (table 9), should not be attributed solely to extrinsic causes as distinct from genetic determination. For, although a "maternal influence" is here probably at work, long-term selection of low sex ratio breeders from first through third, *i.e.*, early litters would tend to concentrate such accessory modifiers of the principal sex-linked gene as might favor the death of affected male embryos in younger but not in older mothers.

Increasing maternal age tended to dilute the low as well as high sex ratio trends selected for in KING's (1918) experiments with rats for 19 successive generations. This fact was not emphasized by KING, but is evident from her extensive tabulations. There are other instances of maternal age effects on gene expression. MACDOWELL, POTTER and TAYLOR (1945) found ad-

vancing parturition age correlated with a progressive delay in the appearance of leukemia in mice with otherwise high incidence. Leukemia in C58 mice is primarily under genetic rather than milk-factor control. Similarly, survival time of p Brunt mice bearing chemically induced fibrosorcomas increased with litter sequence (STRONG 1950a). Induced sarcoma in hybrids between NHO and C57 developed much more slowly in mice of first and second litters than in mice of fifth and sixth litters (STRONG 1948).

Our own results as well as those of KING (1918), MACDOWELL *et al.* (1945), and STRONG (1948, 1950a), reveal maternal age influences of obscure and not necessarily similar nature, the common functional denominator of which is " compensatory " interference with phenotypic manifestation of certain genes.

Thus, the drift toward normal sex ratio in fourth and later litters of our A7 mice may be considered as extrinsically conditioned. The consistent low sex ratio in the first through third A7 litters is, however, unquestionably under gene control. It should be emphasized again that, after more than 90 generations of brother-sister inbreeding, the A-strain is practically isogenic and exhibits normal sex ratio at birth in our sublines A7N and A3, as well as in other laboratories (HESTON personal communication; STRONG 1950b).

Subline A7, derived from the offspring of a single female with low sex ratio mated to her brother, gave a population mean of about 4 daughters : 3 sons during six successive generations of systematic selection. The 4 : 3 mean was not characteristic of individual breeders but was the product of the combined 2 : 1 (lethal) and 1 : 1 (normal) ratios of two classes of A7 females. These two classes were almost identical in size. Females reproducing at the 1 : 1 ratio bred true, as demonstrated by the extraction of normal subline A7N from A7. Females of the 2 : 1 class did not breed true, but produced both normal and " carrier " daughters in about equal numbers. Hence selection had to be continuous during successive generations.

When selection was temporarily interrupted and resumed three generations later (fig. 1) sex ratio behaved accordingly, exhibiting a much steeper temporary trend toward normalcy and return toward the theoretical optimum of 4 : 3 in our F_{10} than would be compatible with multiple factor control of the recorded events. Our results, therefore, warrant the interpretation that half of the females of subline A7 differ from their normal sisters and from the females of control subline A7N and A3 by a recessive sex-linked lethal gene.

The weakest link in our otherwise consistent evidence for a sex-linked lethal is the litter-size paradox (table 7). How can the increase (rather than 25 percent theoretical decrease) in litter size of low sex ratio A7 females as compared with their normal sisters be reconciled with the postulated lethal mechanism? DOBZHANSKY (1950) has stressed that " individual vigor and fecundity are not necessarily correlated, and a superior fecundity may compensate or even overcompensate for deficient vigor . . . A gene *A* may be deleterious in combination with *B*, neutral with *C*, and useful with *D*." It has been suggested to us by DR. J. SCHULTZ that the increased litter size of

females with lethal sex ratio may be a case of heterosis. The genic difference(s) between the normal and the lethal X chromosome in otherwise homozygous mice is (are) perhaps sufficient to account for a hybrid vigor phenomenon resulting in larger litters.

There is at present only one relevant case, showing that a recessive lethal may, indeed, behave in this fashion. STERN and NOVITSKI (1948) have tested the viability of Drosophila females heterozygous for sex-linked lethals. While most of these factors tended to decrease viability of carriers (as compared with flies homozygous for the non-lethal allele), at least 3 of the lethals appeared to enhance viability in several repeated experiments (STERN unpublished data). In other words, improved viability is not incompatible with the presence of a lethal.

If the increased litter size of our A7 carrier mice is due to heterozygosis for the lethal itself, one could further speculate as to pleiotropism of the lethal which is "deleterious" and recessive in its lethal role, but which could be "useful" and exhibit partial dominance in the performance of additional functions ultimately expressed as larger litter size. A variety of pleiotropic effects of autosomal lethals in mice, *e.g.*, the grey-lethal (GRÜNEBERG 1938) and the yellow lethal (DANFORTH 1927; LITTLE 1934; HESTON 1942; HESTON and DERINGER 1947), have been described. A double dose of the A^y gene is, of course, lethal for both sexes. When present in a single dose, yellow exhibits an especially ramified spectrum of neutral, useful and deleterious functions affecting hair color, body size, obesity, hormonal secretion, development and incidence of spontaneous mammary and pulmonary tumors, response to chemical carcinogens. The superficial discrepancy between a lethal effect on the one hand and increased litter size on the other, as found in our A7 mice, can be understood in terms of heterosis and pleiotropism, and does not necessarily conflict with the more positive evidence presented in this report.

SUMMARY

1. A brother-sister mated subline (A7) of the A-strain mouse with an overall sex ratio at birth of approximately 4 females : 3 males has been developed and maintained through eleven successive generations (over 4000 mice) by intermittent selection of breeders from litters with a mean sex ratio of 2 : 1, *i.e.*, 33.3 percent males.

2. The females in this subline were of two distinct classes producing young at the lethal sex ratio of 2 : 1 and the normal ratio of 1 : 1, respectively. A subline with entirely normal sex ratio (A7N) was extracted from the latter category. Males did not transmit the genetic mechanism responsible for the abnormal ratio.

3. A recessive lethal gene, located on the differential segment of the X chromosome of one half of the line A7 females would best account for the results. Statistical analysis of the data shows significant agreement with the expectations under this hypothesis, which is further strengthened by the

anatomical evidence obtained from 51 dissections of pregnant uteri and corresponding corpora lutea. The lethal effect probably occurs after implantation during the first 10 days of gestation.

4. With increasing parturition age, a "maternal influence" appears to counteract low sex ratio in fourth and subsequent litters.

5. The paradoxical increase (rather than 25 percent theoretical decrease) of litter size in litters born by females supposedly carrying the sex-linked lethal may be indicative of pleiotropic action of this gene, or it may be tentatively ascribed to heterosis which could result from the gene difference between the normal and the lethal X chromosome in otherwise homozygous females.

ACKNOWLEDGMENTS

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VARIATION IN THE HOODED PATTERN OF RATS, AND A NEW ALLELE OF HOODED *

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THE earliest recorded studies of color variation in rats were made by CRAMPE in the pre-Mendelian period, 1877–1885, and were published in an important European agricultural journal.

CRAMPE's work was reviewed from a Mendelian point of view by BATESON (1903) and by BATESON's pupil DONCASTER (1906), who also carried out a considerable number of breeding experiments involving the color variations, wild gray, black, hooded, and albino.

Their findings we should now describe as showing the existence of three independent pairs of genes, (1) *C*, colored *vs. c*, albino, (2) *A*, agouti (gray) *vs. a*, non-agouti (black), and (3) *H*, self *vs. h*, hooded.

Contrary to the opinion of DONCASTER, it has since been shown that self (*H*) and Irish (h^i) are distinct dominant alleles of hooded (*h*). Irish has a white spot on the belly between and behind the front legs. This spot is lacking in self, which is pigmented all over. Hooded (known also as piebald) is a pattern in which the entire ventral surface back of the head is white. Dorsally pigment is limited to the head and shoulders (the "hood") and a mid-dorsal stripe extending back to the tip of the tail. The pattern varies chiefly in a quantitative way, as seen in the schematic diagrams shown in figure 1. If the quantitative variation is in a plus direction, the hood grows longer and the back stripe wider. If variation is in a minus direction, the hood grows shorter and the back stripe narrower. The back stripe in extreme cases may become interrupted or disappear completely leaving the only pigmentation located on the dorsal side of the head.

MACCURDY and CASTLE (1907) confirmed the findings of BATESON and DONCASTER as regards the three gene pairs already named. They also undertook a study of variations of the hooded pattern, both when hooded rats are bred without outcrossing and when they are outcrossed to wild gray rats, and hooded individuals are recovered in F_2.

They found that a cross with wild rats tended to increase the pigmented areas, lengthening the hood and widening the back stripe. They estimated the variations in the hooded pattern in percentages of the dorsal surface posterior to the hood which were pigmented, observing a range from 0 percent to 75 percent, equivalent in grades of CASTLE and PHILLIPS to a range from -2 to $+3$. See figure 1 of this paper. They instituted experiments to see if selection of extreme variates, plus or minus, would be effective in changing the racial

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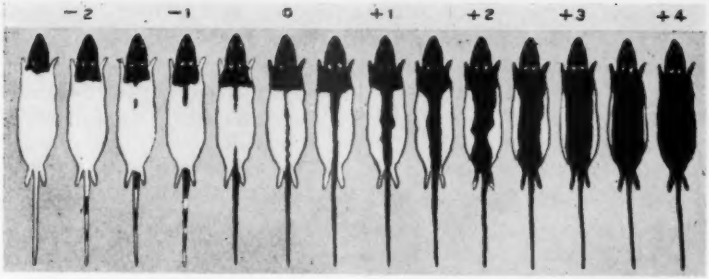

FIGURE 1.—Grading scale for hooded rats. After CASTLE and PHILLIPS (1914).

mean. For this purpose they employed a hooded stock which in three successive generations, including 183 individuals, had a range of variation from 0 to 35 percent (equivalent to grades −2 and +1½, figure 1). Eleven individuals from this stock having a mean grade of 11 percent produced 83 young with a mean grade of 9.6 percent. From these young were selected individuals having a mean grade of 7.8 percent, and from their young were selected parents of mean grade 1.7 percent to produce a third generation. The results of this selection experiment toward less pigmentation posterior to the hood may be summarized thus:

	Mean of parents	Mean of young	Number of young
Gen. 1	11.0	9.6	83
Gen. 2	7.8	5.6	61
Gen. 3	1.7	2.0	24

Selection clearly had been effective in decreasing the pigmentation.

In plus selections parents were chosen of mixed Irish and hooded ancestry. The respective means of parents and young in three different selections were as follows:

	Mean of parents	Mean of young	Number of young
Lot 1	12.0	15.8	111
Lot 2	23.0	22.5	70
Lot 3	40.7	36.2	64

None of these lots was continued into a second generation, but an increase in the grade of the parents is seen to be attended by a like increase in the grade of the offspring, supporting the idea that selection would be effective in changing the grade of the hooded pattern. This idea was admittedly contrary to the view of DE VRIES, then and subsequently widely held, that quantitatively varying characters can not be changed permanently by selection.

Accordingly it was decided to carry the selection experiment farther. This was done by CASTLE and PHILLIPS (1914). They devised a more convenient and practical grading scale shown in figure 1. They took as foundation stock for plus selection the darkest animals left by MACCURDY on completion of his

experiments, and for minus selection the lightest animals left by MACCURDY. The parents employed in starting the plus series ranged in grades from + 1½ to + 3; in the minus series all parents were close to grade – 1½.

The mean grade and the range in variation of the offspring in 16 successive generations of the plus series, and in 17 generations of the minus series are shown in figure 2.

The two series overlapped in range in generation 2, but thereafter their means drew steadily apart and their ranges did not overlap. In generation 16, in the plus series, a population of 1690 rats had a mean grade of + 4.13 and a range of from + 3¼ to + 5¾; in the minus series a population of 1980 rats had a mean grade of – 2.63 and a range from – 1 to – 4.

The difference between the mean grades in the two series amounted to 6¾ grade units. The modal phenotype in the plus series had the back completely black and a small amount of the ventral surface also black. The modal

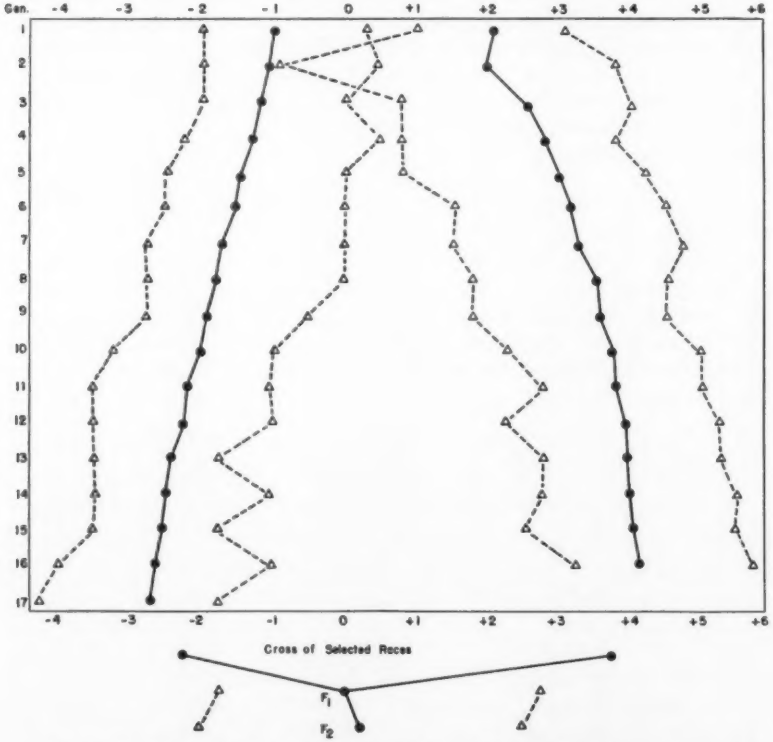

FIGURE 2.—Results of selecting hooded rats toward decreased and increased amounts of pigmentation through 16 successive generations. Heavy lines connect mean grades for each generation, broken lines connect grades of extreme variates in each generation, thus showing range of variation in each series.

type in the minus series had the body completely white, with pigment confined to the dorsal surface of the head.

With such evidence in hand, is it surprising that CASTLE should have thought, as late as 1916, that modification had been effected in the gene for the hooded character?

However, discoveries made subsequent to 1911 had shown that modification of the phenotype of Mendelizing characters may be effected by *other genes* associated with the essential gene for a Mendelian character. Might not this be true also for the gene for hooded in the rat?

CASTLE and PHILLIPS had crossed with each other modal representatives of plus and minus series after 5 or 6 generations of selection separately. The young obtained both in F_1 and F_2 were of intermediate grade, though somewhat more variable in F_2 than in F_1. See bottom of figure 2. They had also from time to time made crosses with wild rats to test the permanency of the modifications made by selection in the hooded pattern, from generation 2 to generation 10. The hooded young extracted from such crosses in F_2 were in the minus series changed from a grade of -2 in the hooded grandparent to a grade of about $+\frac{1}{4}$ in the grandchildren, a total loss of the effects of minus selection amounting to $2\frac{1}{4}$ grades. In the plus series the selected hooded parents were of grade about $+3\frac{1}{2}$, the extracted hooded grandchildren being of practically the same grade, unchanged by a cross with wild.

The difference between the two selected races had accordingly been reduced by a cross with a wild race, principally by a change in the minus race, but the difference still persisted to the extent of about $3\frac{1}{4}$ grades. Would further crossing with wild rats reduce it further, or perhaps abolish it? To test this question CASTLE (1919) made three successive crosses with

TABLE 1

Results of crossing the plus selected race with a wild race.

	Mean grade	Standard deviation	Number of young
Control, uncrossed plus race generation 10	+3.73	0.36	776
Once extracted hooded F_2 young	+3.17	0.73	73
Twice extracted hooded F_2 young	+3.34	0.50	256
Thrice extracted hooded F_2 young	+3.04	0.64	19

TABLE 2

Results of crossing the minus selected race with a wild race.

	Mean grade	Standard deviation	Number of young
Control, uncrossed minus race, generation 16	−2.63	0.27	1,980
Once extracted hooded F_2 young	−0.38	1.25	121
Twice extracted hooded F_2 young	+1.01	0.92	49
Thrice extracted hooded F_2 young	+2.55	0.66	104

wild rats, using an uncrossed plus stock of mean grade + 3.73, and an uncrossed minus stock of mean grade – 2.63. The results are recorded in tables 1 and 2 and shown graphically in figure 3.

The plus selected stock by three successive outcrosses to wild, was made lighter by only about half a grade. But the minus selected stock was made darker by all of five grades. The two stocks were after the third cross with wild just about half a grade apart. Presumably additional crosses would have completely extinguished this small difference.

The logical conclusion to be drawn from this experiment was and is that the progressive changes made in the hooded pattern by 16 successive generations of selection were due wholly to multiple modifying factors which became associated with the gene for hooded, that gene itself having remained meanwhile unchanged. In accumulation of modifiers the minus series had departed more from the wild race than had the plus series.

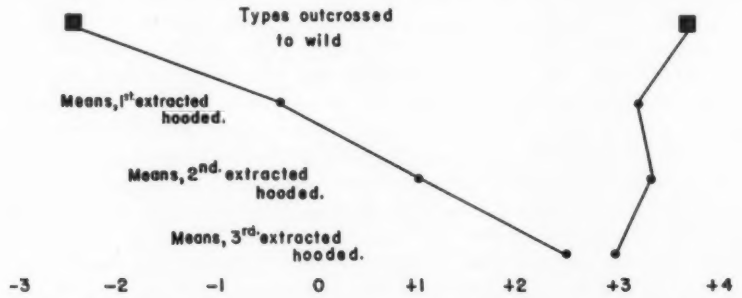

FIGURE 3.—Results of three successive crosses of selected minus and plus rats to wild (self gray) stock, showing the mean grade of the hooded young recovered after each outcross. After CASTLE (1919).

Change of phenotype in a one-gene Mendelizing character was, by the experiment just described, shown to be a matter of accumulation of modifying factors rather than a change in the gene itself. Nevertheless evidence had been obtained incidentally that major changes may take place in the gene itself, altering the phenotype abruptly and permanently. One such mutation observed by CASTLE and PHILLIPS was a mutation from the hooded pattern (h) to the Irish pattern (h^i), in which the entire coat is pigmented except for a white spot of varying size between the fore legs and extending thence backward with increase in its size. This mutation occurred in the tenth generation of the plus selected series, in two individuals, sired by the same male, whose grade was + 4. Both mutant individuals were of much higher grade than their parents, *i.e.*, more nearly black all over. They were graded + 5½ and + 5¾ respectively, + 6 being the grade assigned to an all black rat.

Extensive breeding experiments made with the mutants proved that both were heterozygous (hh^i) and bore associated modifiers of the plus race. For when the mutant male was mated with females of the plus race, half the

young were mutants similar to their sire in grade, the other half being not different from ordinary individuals of the plus series in grade. But when the mutant male was mated with females of the minus race, half the young were mutants of lower average grade than their sire, and the others were much lower in grade than the average of the plus series. This result showed that modifiers introduced from the minus parent lessened the pigmented areas in the offspring, both in the mutant hybrid group (hh^t) and in the homozygous hooded group (hh).

CURTIS and DUNNING (1937) made careful studies of variation in the extent of the hooded pattern in several different inbred hooded races used in cancer investigations. They found that in each race the pattern varied about a characteristic modal grade, differences between them being assumed to be entirely due to modifiers rather than to differences in the gene for hooded pattern. But they also observed two independent instances of mutation from h to h^t, comparable with the case of mutation reported by CASTLE and PHILLIPS.

How frequent in occurrence such mutational changes in the gene for hooded are, we can only conjecture. CASTLE and PHILLIPS observed one in a plus selected population of 8,941 hooded rats. But their minus selected series contained no observed mutation either plus or minus, though it consisted of 11,704 carefully graded hooded rats. So for their entire experiment, the score is one mutation in a population of 20,645. CASTLE and PINCUS (1926) observed one minus mutation (lowering the average grade about ½ grade) in 2062 young. Curtis and Dunning observed two mutations from h to h^t in populations totaling 5,640. Combining all the data, the demonstrated occurrences of mutation in the gene for hooded are four in 28,347, or approximately one in 7,000.

NOTCH, A NEW ALLELE OF HOODED

Quite recently there has been observed a major mutation of the gene for hooded (h) in a minus direction, which it is my purpose to describe as a new allele (h^n), to be called *notch*. See figure 4. Unfortunately I cannot state under what conditions or in what stock it first occurred. My attention was called to it when I observed some rats closely resembling the most lightly pigmented variants of the long extinct selection series, of generations 13–17, reported in 1916. They made their appearance in 1947 as recessives extracted from linkage crosses. They had no connection with the prolonged selection experiment terminated in 1919. Out of curiosity I put them aside and bred them together. Their young varied very closely about a mode of −3, from which they ranged not more than half a grade in either direction. See the graph at the top of figure 7.

Since they looked so much like the final product of a long continued minus selection, I was curious to know whether they too were a mere product of accumulated minus modifying factors, or were due to a mutational change in the gene for hooded itself. To test the matter, I made a cross of a typical −3 male of the new " notch " stock with a typical +2 female of a pink eyed,

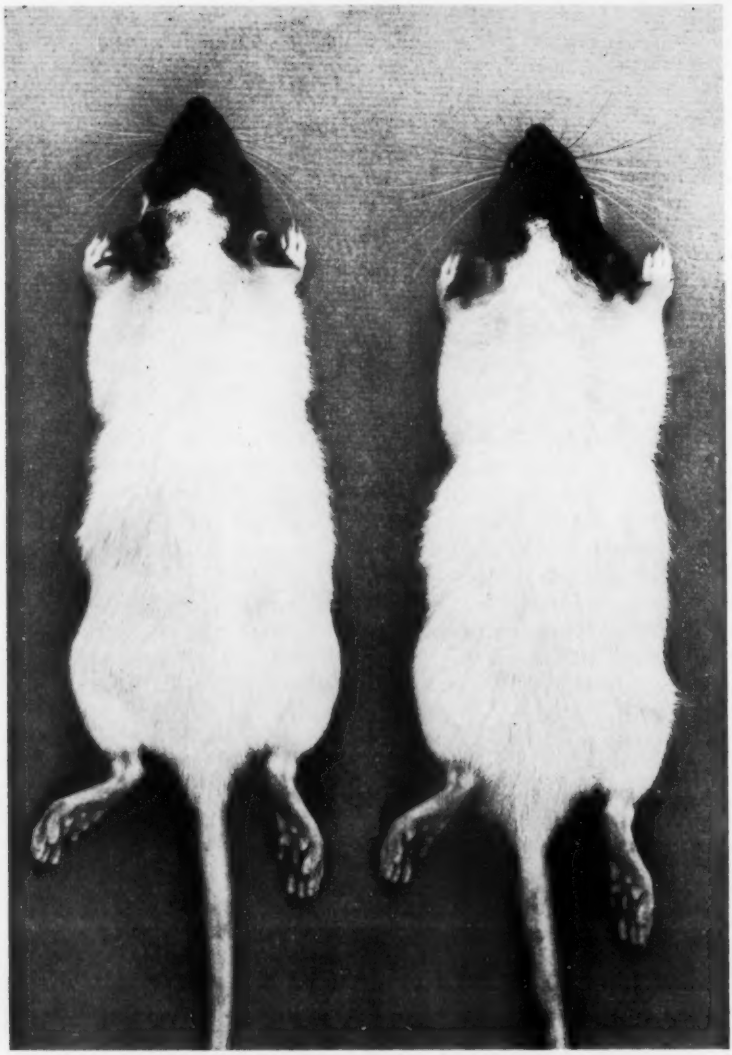

FIGURE 4.—Typical "notch" rats, grade —3.

hooded race, which had been bred brother with sister for four generations and which ranged from +1 to +2½ in grade. See figures 5 and 7.

The resulting litter of F_1 young were all much alike and were graded –1½, being considerably nearer to the notch parent than to the pink-eyed parent in grade. They were back-crossed to notch individuals of grade –3, producing

FIGURE 5.—A pink-eyed hooded rat of grade + 2.

a population of 398 individuals, the grade distribution of which is shown in figure 7.

If the notch and hooded parental phenotypes are due to different alleles of hooded, the formula of the respective parents would be $h^n h^n$ and hh respectively, and the formula of the F_1 individuals would be $h^n h$, modifying factors

FIGURE 6.—A black race (Irish) rat of grade + 5 3/4.

being disregarded. The back-cross mating might be expressed as $h^n h \times h^n h^n$, and would result in equal numbers of $h^n h$ and $h^n h^n$ individuals. The former should resemble the F_1 parent in grade, the latter should resemble the notch parent in grade, except as a possible result of a regrouping of modifying factors.

Included in the undoubted members of the $h^n h$ (hybrid) group would be all individuals to the right of class – 2 which is the largest and most central

of the grade groups. In the $h^n h^n$ group would fall all individuals to the left of class – 2. The former number 107, the latter 176.

The undistributed class of – 2 individuals represents overlapping of the two genotypes. It includes 115 individuals. If we distribute them so as to make the two theoretical genotype groups equal, each group will contain 199 individuals. Thus 23 individuals of the – 2 group will be assigned to the $h^n h^n$

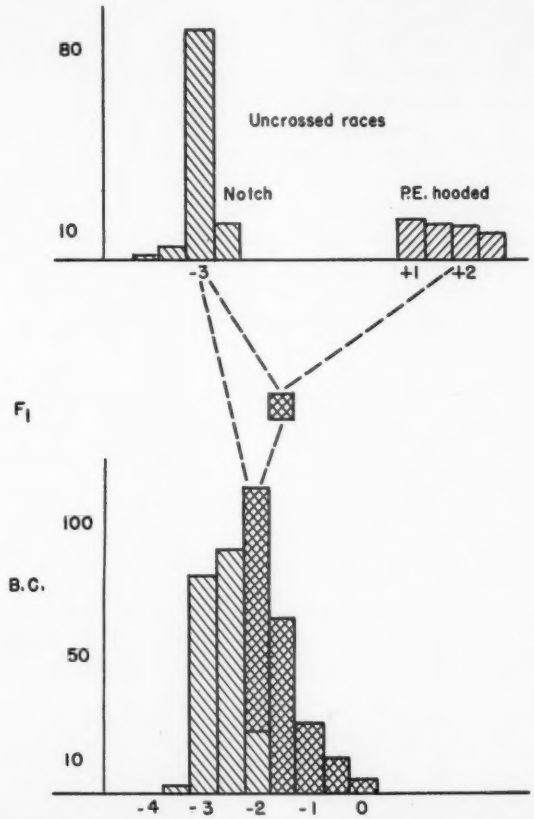

FIGURE 7.—Results of crossing notch with p.e. hooded, and of back crossing F_1 to notch.

genotype and the remaining 92 individuals to the hybrid ($h^n h$) genotype, as indicated in figure 7. The mean grade of the former group will then be – 2.71, which is only a quarter grade less than the mean of the uncrossed notch parental group. The mean grade of the hybrid group ($h^n h$) will be – 1.58, deviating only a trifle from the grade of the parental F_1 group (– 1.50). These slight alterations in mean grade are possibly due to regrouping of modifying

factors in which the two parents may have differed, or they may be without statistical significance.

If we assign the entire -2 class to the hybrid genotype, the averages will be changed only slightly, becoming -2.75 and -1.62 respectively.

We see accordingly that the results thus far obtained support the hypothesis that the difference of more than four grade units between the parental short-hooded and pink-eyed hooded groups was due to a difference in alleles of the hooded gene rather than to a difference in modifying factors.

As a further test of the hypothesis, it was proposed to attempt to equalize the distribution of modifying factors by crossing each race with a third non-hooded race. For this use a black race was employed which had been brother-sister inbred for four generations without the occurrence of any hooded individuals among the progeny. Its phenotype was about $+5\frac{3}{4}$, figure 6 (small ventral white spot) with an occasional $+6$ self black individual. Its genotype may be assumed to be $h^i h^i$, the Irish, small belly-spot allele of hooded.

The mating notch (grade -3) $\times$ black produced young with the belly entirely white, often with a sprinkling also of white hairs low on the sides. Their grade would be close to $+4$. The mating pink-eyed hooded $\times$ black produced young with white belly spots, grading about $+5\frac{1}{2}$.

An F_2 population was produced from each of the crosses, yielding as expected about 25 per cent of hooded individuals.

The hooded individuals obtained from the notch cross (129 in number) had a modal grade of $-2\frac{3}{4}$, average -2.65. See figure 8. They represent the recovered $h^n h^n$ genotype with modifiers derived in part from the black race, in part from the uncrossed notch race.

The hooded young obtained from the pink-eyed hooded cross (numbering 101) represent the recovered hh genotype with modifiers derived in part from the black race, in part from the pink-eyed hooded race. Their modal grade is $+2$, average, $+1.41$.

This result indicates that notwithstanding a partial equalization of modifiers in the two groups of extracted hooded individuals, those groups involve strikingly different alleles of hooded, differing by about four units of our grading scale.

To make assurance doubly sure by further equalization of modifiers, a second outcross to the black race was made of hooded individuals extracted from the first outcross. See figure 8. The individuals selected for the outcross were an extracted notch, grade $-2\frac{3}{4}$, and an extracted hood of grade $+1\frac{1}{4}$.

The hooded young extracted in F_2 from this second outcross numbered 135 in the notch series. They had a modal grade of -2, average -1.98. In the series extracted from the pink-eyed hooded outcross, the young numbered 106, their modal grade was $+2$, average, $+1.95$. See figure 8.

In both series the modifiers should now theoretically be three-fourths derivatives from the black race, one-fourth derivatives from the uncrossed parent race. In both series the second outcross made the extracted hooded group *darker* than it had been after the first outcross. This indicates that modifiers in the black race were more plus in character than those of either uncrossed

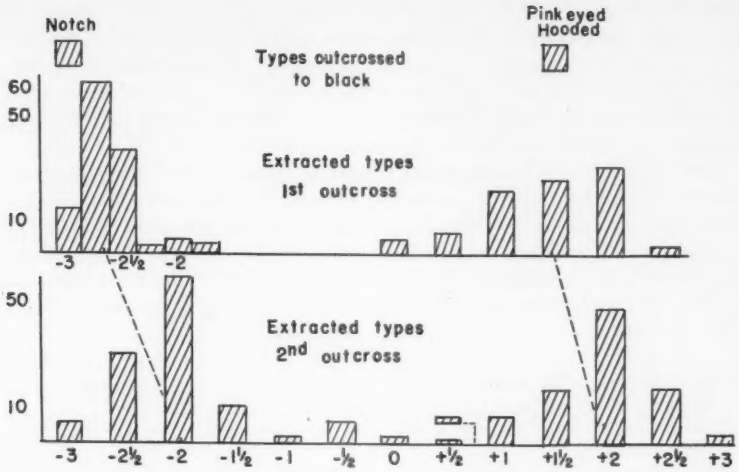

FIGURE 8.—Results of twice out-crossing notch and p.e. hooded to black Irish, and of extracting the hooded variates in F_2. The grade of the extracted hooded in each cross is shown in the figure.

race. This further equalization of modifiers left the two types, notch and hooded, still four grade units apart, very much as they had been in the uncrossed races, though the two had then probably differed somewhat in modifiers. Now they were substantially alike as to modifiers, but still quite distinct, evidently alleles, not modifications of a single genotype, such as were the extreme types secured in the selection experiments of CASTLE and PHILLIPS.

SUMMARY

The hooded pattern of rats is one of the earliest discovered Mendelian unit-characters of mammals and has been studied more extensively than that of any other mammal. It varies in the extent of its pigmented areas through the action of two different genetic agencies. One of these, always present, consists of multiple modifying factors, presumably not themselves capable of producing a coat pattern, but acting as modifiers of the action of the gene for hooded. By selection the total genetic action of these modifying factors may be changed, so as to result either in increased or in decreased areas of pigmentation. With the progress of selection in a definite direction, either plus or minus, variability in the pattern decreases somewhat but never disappears entirely. Presumably the number of modifying factors (genes other than the gene proper for hooded) is very large, or their interactions so complicated, that a condition of complete stability of genetic agencies for pattern production is unattainable.

The gene for hooded pattern (h), a simple recessive in heredity to uniformly pigmented coat of wild rats (H), is subject to rare spontaneous mutations resulting in changed amounts of pigmented areas. Such mutations have

led to the production of the allele h^i (Irish) resulting in a marked increase of pigmented areas, covering the entire body except a small belly spot. Another allele described in this paper for the first time is h^n (notch) effecting a reduction of the pigmented areas equaling or exceeding the most extensive decrease effected by long continued minus selection through the agency of modifying factors.

The incidence of mutation at the *h* locus is indicated to be something like one in 7,000. The alleles of hooded at present demonstrated are *H* self, h^i Irish, *h* hooded, and h^n notch, in an order of decreasing areas of pigmentation.

I am indebted to the Division of Genetics of the College of Agriculture, UNIVERSITY OF CALIFORNIA, and in particular to DR. E. R. DEMPSTER, for facilities generously put at my disposal, for carrying on this investigation.

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NON-RANDOM DISJUNCTION IN DROSOPHILA[1]

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THE stability of the ring chromosomes in *Drosophila melanogaster* raises certain questions about the nature of chromosome structure and reduplication, since it seems possible that such chromosomes would be subject to loss by interlocking of daughter chromatids after reduplication. In a set of experiments designed to determine the extent to which newly derived ring chromosomes might be lost at the meiotic divisions, it has been found that the generalization that disjunction of chromatids at the second meiotic division is random does not appear to be completely valid when the chromatids are structurally different. The evidence for such non-randomness is presented below.

EXPERIMENTS INVOLVING ATTACHED X CHROMOSOMES

The type of genetic constitution which regularly manufactures ring chromosomes is that used by SIDOROV, SOKOLOV and TROFIMOV (1935, 1936) and by STURTEVANT and BEADLE (1936) in their demonstration of single crossing over within heterozygous inversions. The essential features of their analyses of the results of crossing over in this type of tetrad are incorporated into figure 1. At the reduction division the X-chromatids separate from the Y-chromatids; the figure shows only the second division segregation for the X-chromatids. The tetrads with no, one, or two exchanges are represented by the symbols E_0, E_1 or E_2, respectively. The two exchange tetrads (E_2) may involve 2, 3, or 4 strands and are designated as E_2-2s, E_2-3s and E_2-4s. It is to be noted that there are two different genetic consequences from the single exchange tetrads (E_1), and two from each of the three types of two exchange tetrads (E_2-2s, E_2-3s and E_2-4s). These different possibilities are distinguished by the letters a and b following the tetrad type. Three exchange tetrads are not considered here since, as will be shown below, they are relatively rare and can contribute little to the analysis.

From figure 1 it is clear that 50 percent is the maximum frequency with which rings may be expected, regardless of the distribution of tetrads of the different ranks since the no-exchange tetrads give rise only to attached X's and the two-exchange tetrads to 50 percent more attached X's than rings in the viable X-chromosome-bearing gametes.

Neither the work of SIDOROV, SOKOLOV and TROFIMOV nor that of STURTEVANT and BEADLE suggests any deficiency of the ring class. In the first case,

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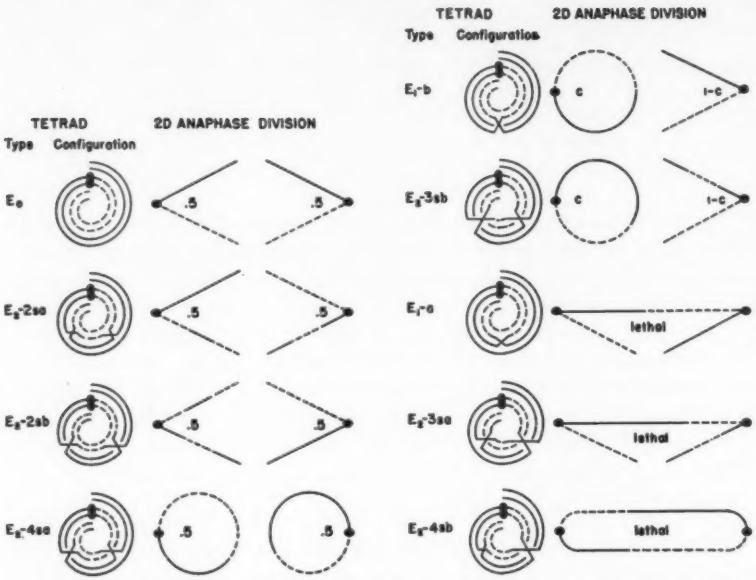

FIGURE 1.—Genetic consequences of no, one and two exchanges in attached X chromosomes heterozygous for an inversion. Fragments resulting after exchange are not diagrammed. Symbols are explained in the text.

the ratio reported is 824 attached X chromosomes to 1084 ring X chromosomes, and in the second 316 attached X chromosomes to 337 rings. It is a striking peculiarity of both sets of data that the frequency of rings exceeds 50 percent. SIDOROV, SOKOLOV and TROFIMOV, recognizing this discrepancy, make a viability correction which brings the percentage of rings down to 54 and suggest further that some undetected superfemales, phenotypically like the ring-bearing class, may have been confused with that class. The data of STURTEVANT and BEADLE, while less extensive, appear equally inconsistent with the tetrad analysis since (a) they conclude, as a best estimate of tetrad frequency, that $E_0 = .048$, $E_1 = .908$ and $E_2 = .044$, which should have given 294 rings to 359 attached X's and (b) the nature of their attached X chromosome, heterozygous for the y^4 inversion, was such as to produce a euchromatic duplication and deficiency ring chromosome which might have an adverse viability effect on the ring-bearing class.

The bearing of this type of experiment on the question discussed in the introduction and the incompatibility of the published data with the simplest type of tetrad analysis has led to a repetition of this experiment using an improved method. To circumvent viability complications, it was necessary to synthesize an attached X chromosome, heterozygous for an inversion, which could, by crossing over, give rise to ring chromosomes viable in the male, *i.e.*,

without any appreciable duplications or deficiencies. The method of doing this is described in detail elsewhere (NOVITSKI and LINDSLEY 1950); it consists essentially of combining, in a 3N female, a ring chromosome with good male viability (X^{c2}, *cv v f*), a chromosome with normal sequence (*y Hw*) and an inverted chromosome (*In* (1) *sc*^{8}, *f y*, *In* (1) EN), and extracting the double crossover in which one of the strands of the ring has crossed over with a chromatid of each of the other chromosomes, thus producing an attached X chromosome effectively heterozygous for an inversion. The crossover product ring from this chromosome is structurally like the X^{c2} chromosome introduced initially into the 3N parent. If a period is used to designate the position of the centromere, the attached X chromosome synthesized in this way carries the mutant genes and their normal alleles in the following order, *y Hw cv*$^{+}$ *v f* · *y*$^{+}$ *Hw*$^{+}$ *cv v*$^{+}$ *f*, and will be referred to as *Hw f* for the sake of brevity.

The F_1 of the cross *Hw f* ♀ ♀ × *sc*^{81} *B In S w*^{a} *sc*^{8} (Muller-5) ♂ ♂ are given in column A of table 1. Since the male parent did not carry any of the mutants found in the derived rings, the female ring-bearing F_1 are listed only in the total row. The ring chromosome carrying *f* but none of the other recessive mutants must have arisen in a three-exchange tetrad; the low number, 1,

TABLE 1

F_1 of y Hw v f. cv f♀♀ × sc^{S1} *B InS w*^{a} *sc*^{8}♂♂*(A) and × sc cv v f B*♂♂*(B)*
Region I, y-cv; Region II, cv-v; Region III v-centromere.

X chromosome constitution	Phenotype	A	B	A + B
Attached X, non crossover	*Hw f* ♀♀	350	624	974
Attached X, crossovers in regions II and III	*Hw v f* ♀♀	17	52	69
Attached X, crossovers in regions I and III	*Hw cv f* ♀♀	30	19	49
Total attached X		397	695	1092
Ring X chromosomes				
Crossovers in region I	*v f B* ♀♀	....	178	
	v f ♂♂	99	215	314
Crossovers in region II	*cv v f B* ♀♀	....	477	
	cv v f ♂♂	242	542	784
Crossovers in region III	*cv f B* ♀♀	....	432	
	cv f ♂♂	240	472	712
Crossovers in regions I, II and III, from 3 exchange tetrads	*f B* ♀♀	....	3	
	f ♂♂	1	1	2
Ring bearing ♀♀	*B* ♀♀	730	....	1820
	(cv) (v) f B ♀♀	....	1090	
Ring bearing ♂♂	*(cv) (v) f* ♂♂	582	1230	1812
Total ring classes		1312	2320	3632
Patroclinous ♂♂	*B w*^{a} ♂♂	1883	....	5488
	sc cv v f B ♂♂	....	3605	

compared to the total is considered ample justification for disregarding tetrads of rank higher than two in the analysis.

Since half of the attached X gametes are lost by fertilization with an X-bearing sperm whereas all the ring chromosome gametes can be recovered, the attached X class must be multiplied by two for comparison with the total of the ring class or, alternatively, the attached X class may be compared directly with either the male or the female ring class. The data indicate quite clearly that there is an excess, well beyond 50 percent, of the ring class. An additional set was run in which the *Hw f* ♀ ♀ were mated to *sc cv v f B* ♂ ♂ ; the results are given in column B of table 1. In this case all the F_1 are homozygous or hemizygous for *f* and the female progeny carrying the ring, as well as the male, also carry other markers so that any viability complications arising from an inequitable distribution of mutant characters should decrease the frequency of the ring classes to a greater extent than the attached X classes. Here, as before, there is an excess of rings, the ratios calculated in the way suggested above being 1390 attached X to 2320 rings, 695 attached X to 1090 rings or 695 attached X to 1230 rings. It should also be noted here that the suggestion of SIDOROV, SOCOLOV and TROFIMOV that undetected superfemales contribute to the excess is invalid in this experiment since they would have a unique phenotype, *Hw f B*, even if none of the typical superfemale characteristics were obvious.

It seems unquestionable that the discrepancy is real and that, therefore, the tetrad analysis presented earlier is in some way defective. The effects of relaxing certain of the assumptions inherent in the analysis will be considered briefly. The assumption of sister strand crossing over in a tetrad analysis reduces the calculated number of no-exchange tetrads (WEINSTEIN 1936). The present problem centers around an observed deficiency of attached X chromosomes, which can be considered mathematically as arising from a negative number of no-exchange tetrads, and the further reduction of this number by the assumption of sister strand crossing over would enhance rather than alleviate the difficulty in interpretation.

Secondly, it has been assumed that the first division is reductional and the second equational. If the opposite were the case, even if only in a proportion of all tetrads, the chromatid bridges shown in figure 1 which are assumed to give rise to lethal zygotes, following STURTEVANT and BEADLE (1936), would instead be first anaphase bridges insuring the passage of a Y chromosome to the functional egg nucleus. As pointed out by the above workers, this would increase the number of patroclinous males recovered but would not alter the theoretically expected proportions of attached X or ring chromosomes. They conclude that, since the ratio of patroclinous males to total females approaches but does not exceed a 2:1 ratio (the value given by the data in table 1 is 1.88:1), second division bridges must be lethal. It may be added, as a corollary to this, that in no appreciable proportion of the tetrads can the first division have been equational rather than reductional.

Finally, it has been taken for granted that the two-, three- and four-strand

types of two exchange tetrads occur in the ratio of 1:2:1, *i.e.*, there is no chromatid interference. From figure 1 it is obvious that any excess of rings over attached X's would have to come from one of the four-strand double classes, E_2-4sa. It is possible to calculate the relative frequencies of the three types of two exchange tetrads (the nature and extent of chromatid interference) necessary to account for the observed excess of ring chromosomes from the data in table 1. Attached X chromosomes (XX) bearing gametes arise from no exchange, two-strand double exchange, and a quarter of the single- and three-strand double exchange tetrads, and are recovered as viable zygotes in that half of the cases where the egg is fertilized by a Y-bearing sperm:

$$2XX = E_0 + E_2\text{-}2s + \tfrac{1}{4}\, E_1 + \tfrac{1}{4}\, E_2\text{-}3s.$$

Ring chromosomes (X^c) are derived from a quarter of the single and three-strand double exchange tetrads, and from half of the four-strand double exchanges:

$$X^c = \tfrac{1}{4}\, E_1 + \tfrac{1}{4}\, E_2\text{-}3s + \tfrac{1}{2}\, E_2\text{-}4s.$$

Furthermore the total of the X-chromosome-bearing gametes should equal the number of Y-chromosome-bearing gametes which may be estimated by doubling the frequency of patroclinous males (Pat ♂ ♂):

$$2\ \text{Pat}\ ♂\ ♂ = E_0 + E_1 + E_2\text{-}2s + E_2\text{-}3s + E_2\text{-}4s.$$

If the values of XX, X^c and Pat ♂ ♂ (1092, 3632 and 5488 respectively) from table 1 were the consequences of regular segregation, the above equations would be satisfied by the following equalities:

$$\begin{aligned} E_2\text{-}4s &= 4208 \\ E_1 + E_2\text{-}3s &= 6112 \\ E_0 + E_2\text{-}2s &= 656. \end{aligned}$$

However, since in ordinary crossover experiments all the four-strand double exchange tetrads and half of the single and three-strand doubles give rise to gametes carrying single crossover chromatids, such an array of tetrads would give rise to $(4208 + \tfrac{1}{2} \cdot 6112)/(4208 + 6112 + 656)$ or 68 percent recombination between X chromosome genes at the extremes of the chromosome. This value is clearly contradicted by the failure of recombination to exceed 50 percent in such experiments.

From the above it seems reasonable to suppose that the appropriate solution is consistent with the usual assumptions of conventional tetrad analyses, lack of sister strand crossing over and chromatid interference, but that after the ring chromosomes are formed by crossing over, more of them get into the functional egg nucleus than do the attached X chromosomes. Such a non-randomness of segregation might be visualized as operating in the following way: During the first anaphase, as the two X-chromatids progress towards the presumptive region of the functional egg nucleus, an orientation of the dyad is set up in which the ring chromatid, perhaps because of its smaller size, tends to occupy a position away from the first metaphase plate. At the second division, which immediately follows the first, the ring may then be advan-

tageously located for inclusion in the female oötid. The orientation of the dyad assumed responsible for determining the chromatid to be included in the functional egg nucleus may be more specifically defined as an orientation of the centromere region. The questions arising from this consideration appear to be similar to those involved in the directed second division segregation of normal chromatids by first anaphase bridges produced by single crossing over within inversion heterozygotes (STURTEVANT and BEADLE 1936) where the chromatid bridge orients the centromere region of the dyad prior to the second division (CARSON 1946).

EXPERIMENTS WITH ROD CHROMOSOMES

Non-randomness resulting from competition of structurally different chromatids for inclusion in the functional egg nucleus might be limited to the sort of experiment described previously but could be a more general property of the meiotic process in Drosophila. There are several general types of chromosome combinations that give rise, by crossing over, to dissimilar chromatids: translocation heterozygotes, duplication and deficiency heterozygotes. These, as a rule, produce also inviable zygotes or involve chromosomes of low viability which may lead to certain ambiguities in interpretation. The experiments to be described here make use of a heterochromatic deficiency for *bb* and block A (*Bk A*) of the X chromosome. The deficiency for *Bk A* has been reported to have no effect on the viability or fertility of an otherwise normal male, or when homozygous in the female, but decreases the length of the metaphase X chromosome by about a third (MULLER and GERSHENSON 1935; MULLER, RAFFEL, GERSHENSON and PROKOFYEVA-BELGOVSKAYA 1937).

The chromosomes used are those synthesized by RAFFEL and MULLER (1942) by combining the right and left ends of certain scute inversions, *In* (1) sc^8, *In* (1) sc^4, and *In* (1) sc^{81}. These inversions have been analyzed in detail by the above workers and have been shown to have the following characteristics: The left breakpoint of the inversion is to the left of the scute gene in the case of *In* (1) sc^8 and to the right in the other two; the right breakpoint is to the left of *bb* and *Bk A* in *In* (1) sc^4 and to the right in the other two. The crossover product $sc^4\ sc^8$ carries a scute gene at the base as well as the tip but is deficient for the heterochromatic region including *bb* and *Bk A*. The $sc^{81}\ sc^8$ combination, like the sc^8 chromosome, has a scute gene at the base and the heterochromatic region including the *bb* locus and *Bk A* at the tip. The essential difference between the two types of chromosomes is that $sc^4\ sc^8$ lacks the heterochromatic region, whereas both sc^{81} and sc^8 carry it at the end and so are physically longer.

The initial cross consisted of females of the compositions *y* sc^4 *car m* w^a sc^8/*In* (1) *dl-49, y w* lz^s and sc^{81} *car m* w^a/*In* (1) *dl-49, y w* lz^s mated to sc^8 *f v cv* males. F_1 ♀ ♀ of the genotype sc^8 *f v cv*/*In* (1) *dl-49, y w* lz^s were mated to y^2 *cv v f* ♂ ♂; their progeny consisted of 674 *cv v f* ♀ ♀, 781 *y* ♀ ♀, 609 *cv v f* ♂ ♂ and 414 *y w* lz^s ♂ ♂. Since all classes should have been equal in

frequency, the inequalities give a measure of the effect of the mutant genes in the two sexes in decreasing the viability. There were 86 percent as many *cv v f* ♀ ♀ as *y* ♀ ♀ and 78 percent as many *cv v f* ♂ ♂. In the crossover experiments below, the complementary crossover types counted as females carry less extreme combinations and therefore the figure of 86 percent should represent the maximum deviation that can be attributed to inviability.

The progeny of the cross *y sc*^{4} *car m w*^{a} *sc*^{8}/*sc*^{8} *f v cv* ♀ ♀ × *y*^{2} *cv v f* ♂ ♂ are given in table 2. The crossovers are classified separately for sex and for the presence of yellow, which marks the shorter deficient chromosome. The identifiable classes are more numerous in the male progeny than the female, being 1 (1′ + 1″), 2 (2′ + 2″) and 3 in the female and in some instances classification of *car* was not possible because of the presence of *w*^{a} and *v*. In those cases a vertical arrow through the classes points to the more general class for which the identification was unambiguous. A dash indicates that that class could not have been identified in any case. A question mark, as in the 3,4 yellow crossover type, indicates an ambiguous class; in this instance such crossovers would probably have been included in the 2″ class.

TABLE 2

Experiment C. F_1 *of* $\frac{y\ sc^4\ 1'\ car\ 1''\ 2'\ m\ 2''\ 3\ 4\ w^a\ sc^8}{Het \qquad\qquad f \qquad\qquad v\ cv \qquad sc^8}$ ♀♀ × *y*^{2} *cv v f* ♂♂. *The abbreviation "Het" refers to the heterochromatic block missing in the sc*^{4} *sc*^{8} *combination chromosome. Other symbols are explained in the text.*

Crossover regions	*y* ♂♂	Het ♂♂	*y* ♀♀	Het ♀♀	Crossover regions	*y* ♂♂	Het ♂♂	*y* ♀♀	Het ♀♀
0	648	344	771	435	1′, 2′	↓	3		
					1″, 2′		1		
1′	67	↓	↓	↓	1 , 2′	9	4		
1″	89				1″, 2″	4	2		
1	156	82	164	91	1′, 2″	2	0	↓	↓
2′	274	118	↓	↓	1 , 2	15	10	9	6
2″	29	13			2′, 4	18	8	....	....
2	303	131	305	167	2″, 4	0	2	....	....
3	206	90	194	97	2′, 3	14	10		
4	44	32	....	....	2″, 3	0	1	↓	↓
					2 , 3	14	11	22	17
1′, 4	↓	2	....	....	3 , 4	?	3	....	....
1″, 4		5	....	....	2′, 2″	2	2	....	....
1 , 4	16	7	....	....	1″, 2′, 4	1	0		
1′, 3	↓	7	↓	↓	1′, 1″, 3	1	0		
1″, 3		11			1′, 2′, 4	1	0		
1 , 3	21	19	35	24	1′, 2′, 3	1	0		
					1 , 2″, 3	0	1		

Simple inspection is sufficient to show that there is a pronounced excess of progeny carrying the smaller chromosome regardless of crossover class or sex, and that this excess is well beyond any expectation formulated in terms of viability effects. The female crossovers in region 1, for instance, give a ratio of 164/91, yet the larger class is homozygous for the mutants *y, cv, v* and *f*, the smaller wild-type.

A different picture is presented by the cross of $sc^{81}\ sc^{8}/sc^{8}$ ♀ ♀ × $y^{2}\ cv\ v\ f$ ♂ ♂ (table 3). Class for class, the ratios vary around 1 : 1. This is not unexpected since both chromosomes carry the heterochromatic region at the ends. This cross constitutes an additional control on the preceding one and shows that the deviations from a 1 : 1 ratio in that case must be a function of the left end of the $sc^{4}\ sc^{8}$ combination chromosome.

TABLE 3

Experiment D. F_1 of $\frac{Het\ car\ 1^{II}\ 2^{I}\ m\ 2^{II}\ 3\ 4\ w^{a}\ sc^{8}}{Het \qquad f \qquad v\ cv \qquad sc^{8}}$ ♀♀ × $y^{2}\ cv\ v\ f$ ♂♂.

Crossover regions	*car* ♂♂	non-*car* ♂♂	*f* ♀♀	non-*f* ♀♀	Crossover regions	*car* ♂♂	non-*car* ♂♂	*f* ♀♀	non-*f* ♀♀
0	296	323	391	378	$1^{II}, 4$	?	4	....	....
					$1^{II}, 3$	?	3	....	....
1^{II}	30	24	....	....	$2^{I}, 4$	11	6	....	....
2^{I}	107	74	↓	↓	$2^{II}, 4$	0	1	....	....
2^{II}	11	22			$2^{I}, 3$	16	10	↓	↓
2	118	96	150	157	$2^{II}, 3$	0	1		
3	87	108	102	119	2 , 3	16	11	17	7
4	27	24	....	....	$2^{I}, 2^{II}$	?	1	....	....

INTERPRETATION OF THE ROD CHROMOSOME RESULTS

The tetrad analysis for the $sc^{4}\ sc^{8}/sc^{8}$ combination is diagrammed in figure 2. It is conventional in all respects except that it is assumed that when the two structurally different chromatids separate at the second meiotic division the probability that the shorter will be included in the functional egg nucleus is not .5 but c, with the probability for the longer being $(1-c)$. Reference to figure 2 reveals that non-randomness of this type would be effective only in the single exchange tetrads (E_1) and the three-strand doubles (E_2-3sa and E_2-3sb). The frequency of each type of recovered strand may be found by summing the proportions of each rank of tetrad giving rise to that type. The abbreviations non, sgl and db are used to indicate non-crossover, single and double crossover strands, respectively; the superscript $^{-}$ designates those recovered strands having the heterochromatic deficiency (*i.e.*, the shorter chromosome) and the superscript $^{+}$ the contrary class. Absence of a superscript represents the sum of the two classes. In the absence of chromatid interference E_2-2s = E_2-3sa = E_2-3sb = E_2-4s = ¼ E_2, then

$$\begin{aligned}
non^{-} &= \tfrac{1}{2}E_0 + \tfrac{1}{2}cE_1 + \tfrac{1}{8}cE_2 + 1/16\,E_2 \\
non^{+} &= \tfrac{1}{2}E_0 + \tfrac{1}{2}(1-c)E_1 + \tfrac{1}{8}(1-c)E_2 + 1/16\,E_2 \\
non &= E_0 + \tfrac{1}{2}E_1 + \tfrac{1}{4}E_2 \\
sgl^{-} &= \tfrac{1}{2}cE_1 + \tfrac{1}{4}cE_2 + \tfrac{1}{8}E_2 \\
sgl^{+} &= \tfrac{1}{2}(1-c)E_1 + \tfrac{1}{4}(1-c)E_2 + \tfrac{1}{8}E_2 \\
sgl &= \tfrac{1}{2}E_1 + \tfrac{1}{2}E_2 \\
db^{-} &= 1/16\,E_2 + \tfrac{1}{8}cE_2 \\
db^{+} &= 1/16\,E_2 + \tfrac{1}{8}(1-c)E_2 \\
db &= \tfrac{1}{4}E_2
\end{aligned}$$

From the equations for non, sgl and db it is clear that the aberrant distribution of crossover types does not affect the usual tetrad analysis provided only that the complementary classes are added together. Table 4 sums the data for the scute crosses; the five triple crossovers have been entered as doubles since the overall frequency of three exchange tetrads must be negligible. The frequencies of tetrads of different ranks have been calculated individually for the male and female classes, using the equations for the sums of the complementary types. With the tetrad values given, it is possible to

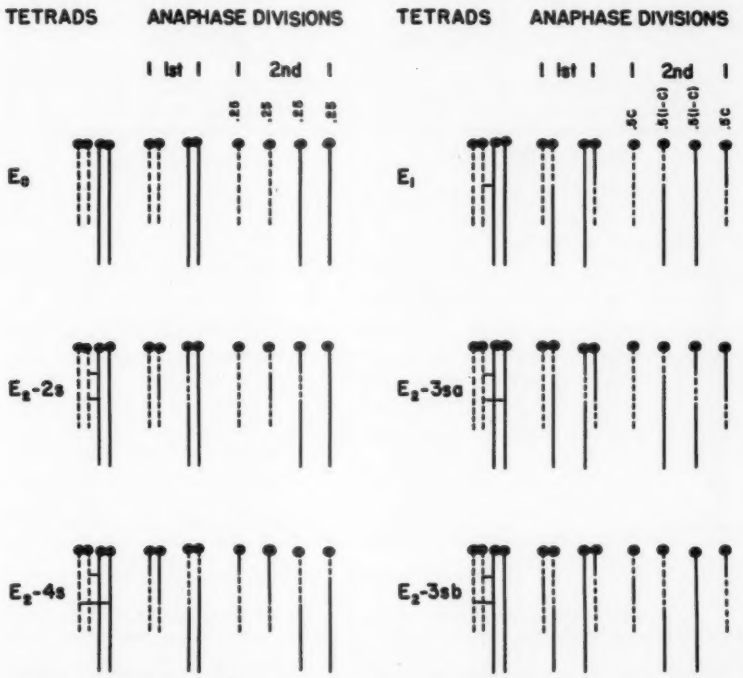

FIGURE 2.—Results of exchange in rod X chromosomes, one of which is deficient for part of the heterochromatic region. The symbols are the same as in figure 1.

calculate the value of c from six independent sets of values (complementary classes give, of course, identical values since the sum of those classes was used for calculating tetrad frequencies). These values listed in table 4 under column c range from .67 to .72, a rather remarkable and undoubtedly to some extent coincidental agreement. Nevertheless, they show quite clearly that the observed deviations, even in the non-crossover class, are accountable for on the assumption that the shorter chromosome is included in the functional egg nucleus about twice as frequently as the longer.

Similar calculations for the $sc^{81}\ sc^{8}/sc^{8}$ cross are presented in table 4. Certain of the double crossover classes are not determinable; a correction has been made by adding seven to this class from the single crossovers, since the ratio of the complementary classes from the other doubles is 1:1 (35/35) and there are seven flies in the complements of the ambiguous classes. The chromosomes carrying the sc^{81} end are entered in the $^{-}$ rows. The derived values of c are aberrant (.35 and .92) when calculated from the calculated double crossover classes of both sexes; the low number of individuals in these classes is undoubtedly responsible for these values. For the non-crossovers and singles, however, they range from .47 to .53, *i.e.*, segregation is random.

TABLE 4

Summation of the non-, single and double crossovers from tables 2 and 3, with the calculated frequencies of tetrads of different rank, and the values for c.

	$sc^{4}\ sc^{8}/sc^{8}$				$sc^{51}\ sc^{8}/sc^{8}$			
	♂		♀		♂		♀	
	N	c	N	c	N	c	N	c
non^{-}	648	.68	771	.70	296	.47	391	.51
non^{+}	344		435		323		378	
non	992		1206		619		769	
sgl^{-}	709	.71	663	.67	262	48	276	.53
sgl^{+}	335		355		245		252	
sgl	1044		1018		507		528	
db^{-}	90	.72	66	.67	25	.35	7	.92
db^{+}	58		47		34		17	
db	148		113		59		24	
Total	2184		2337		1185		1321	
E_2	592		452		236		96	
E_1	1496		1584		77P		960	
E_0	96		301		171		265	

ANALYSIS OF THE ATTACHED X CHROMOSOME RESULTS

The above formulation may be applied to the tetrad analysis of the attached X chromosome heterozygous for an inversion. The tetrad frequencies will refer here only to that half of the gametes receiving an X centromere, although the estimate of the total number of such gametes comes from the number of patroclinous males, that is, those receiving a Y chromosome. There are four distinguishable classes, the total of females with attached X's, those with attached X's equational for one or more of the heterozygous mutants, ring-bearing males and females and patroclinous males, to be abbreviated as XX, c-o XX, X^{e}, and Pat ♂ ♂, respectively. From figure 1 it can be seen that

$$XX = E_0 + (1-c)E_1\text{-b} + E_2\text{-2s} + (1-c)E_2\text{-3sb}.$$
$$X^{e} = cE_1\text{-b} + cE_2\text{-3sb} + E_2\text{-4sa}$$

Equationals are detected only in half the cases of homozygosis, so

$$\text{c-o XX} = \tfrac{1}{2}\,E_2\text{-2sa} + \tfrac{1}{2}\,(1-c)E_2\text{-3sb}$$

and the number of Y-bearing gametes recovered as patroclinous males after fertilization by an X-bearing sperm should equal one-half those receiving X-chromatids, or

$$\text{Pat } ♂\,♂ = \tfrac{1}{2}\,(E_0 + E_1 + E_2)$$

Furthermore, $E_1a = E_1b = \tfrac{1}{2}\,E_1$, E_2-2sa = E_2-2sb, E_2-3sa = E_2-3sb, E_2-4sa = E_2-4sb and in the absence of chromatid interference, E_2-2s = E_2-3sa = E_2-3sb = E_2-4s = $\tfrac{1}{4}\,E_2$.

A very convenient expression for c is given by

$$c = \frac{X^c}{2\text{ Pat } ♂\,♂ - XX - X^c} - \frac{E_2\,(1-c)}{4E_1 + 3E_2}$$

(The derivation of this solution is tedious; its validity may be demonstrated, however, by substituting for Pat ♂ ♂, XX, and X^c the terms given in the initial equations.)

E_2 is somewhat smaller than E_1 in the usual tetrad analysis (see, for instance, the values calculated for E_1 and E_2 from the scute inversion experiments). Disregarding the last term of the equation in computing c leads to an overestimate which equals zero when c is one, becoming progressively larger as c decreases and amounting perhaps to as much as five percent when $c = .5$. The extent of the error is insignificant compared to the inherent variability of the raw data. The advantage to the simplified equation is that it does not involve the number of the crossover attached-X's, a class which is small, difficult to determine accurately and, for the data collected by SIDOROV, SOKOLOV and TROFIMOV, not available.

In determining c, the observed number of XX progeny must be doubled since a half are lost by fertilization by an X-bearing sperm, and when the derived ring chromosome is lethal in the male, as it is in experiments of SIDOROV, SOKOLOV and TROFIMOV, and of STURTEVANT and BEADLE, that figure must also be doubled. The calculated values are given in table 5.

TABLE 5

Determination of c from the attached-X experiments.

Experiment of	2 Pat ♂♂	2XX	X^c	c
STURTEVANT & BEADLE	2196	632	674	.76
SIDOROV, SOKOLOV & TROFIMOV (1936)	7062	1648	2168	.67
Experiment A	3766	794	1312	.79
Experiment B	7210	1390	2318	.66

DISCUSSION

The possibility that newly formed ring chromosomes may be lost in some fraction of all cases by an interlocking of daughter chromatids after the first reduplication cannot be denied nor confirmed from the experiments with an attached X heterozygous for an inversion. The degree of non-randomness of disjunction (c) necessary to account for the observed data has been calculated

by assuming no loss; it seems evident that a higher value for c with some loss of ring chromosomes would be equally compatible with the data. Although the information from the two kinds of experiments, with attached X's and with the scute inversions does not give striking differences in the value of c, it seems reasonable to suppose that the magnitude of the competition between structurally dissimilar chromatids must be some function of the degree and kind of dissimilarity.

As mentioned previously, a number of different kinds of genetic constitutions should give rise to asymmetrical dyads at the second meiotic division, with subsequent non-random disjunction. A number of such instances can be found in previously published work and may be considered briefly here. MATHER (1939) mentions that "the sc^8 deficiency chromosome requires further comment . . . (it) shows a very peculiar type of segregation from normal sc^8 chromosomes. In the daughters of a sc^8/sc^8 def. female the deficiency is recovered just twice as frequently as the corresponding portion of the other chromosomes. . . . The reasons for this behavior are not fully known, but it seems to be bound up with a maternal effect." Further, STURTEVANT and BEADLE (1936), from a similar experiment found a total of 186 sc^8 progeny and 133 sc^8 deficiency progeny. The sc^8 deficiency chromosome is lethal in the male, unlike the $sc^4\ sc^8$ combination chromosome used in the experiments described in this paper; the deviation from the 2:1 ratio expected, (♀♀+♂♂):♀♀, is significant ($P < .002$) but agrees with $sc^4\ sc^8/sc^8$ experiment ($P = .25$) despite a distribution of mutants in their experiments which should decrease the deficiency class disproportionately and the existence of one highly aberrant crossover class (the complementary types of crossovers in region 4 were in the ratio of 29 sc^8 chromosomes to 2 sc^8 def. chromosomes) tending to obscure the extent of the discrepancy.

The bearing of the suggestion of non-random separation on the interpretation of segregation in translocation heterozygotes is well illustrated by the data presented by GLASS (1934, 1935). Three different translocations, each involving a breakpoint at the right end of the second chromosome and one near the centromere of the third, were studied, in the heterozygous state, with respect to the frequencies of different combinations produced. GLASS points out that ". . . one of the complementary aneuploid classes is almost twice the size of the other" and further tests show that ". . . the phenomenon is not one of differential viability of the classes; nor can it be due either to a gametic lethal, or to lethality of gametes carrying a particular type of chromosomal abnormality." He concludes that possibly "the presence of ordinary recessive lethals, by crossing over, eliminates the non-disjunctional zygotes carrying them in homozygous condition," but points out that the assumption of maximum crossing over could be responsible for a loss only one-third as great as that observed. The fact that all three translocations show precisely the same effect when heterozygous (V_3/+, 279/151; V_4/+, 1242/709 and V_5/+, 246/125) can be considered fairly strong evidence that the accidental occurrence of a recessive lethal in the translocation stocks is not responsible for the approximate 2:1 ratio of the aneuploid types.

Interpretation on the basis of non-random disjunction is not only consistent with the experiments reported above, but, in fact, leads to a simplified concept of the nature of the segregation process in these translocation heterozygotes. Let 2L · 2R and 3L · 3R represent the normal second and third chromosomes, respectively, 2L · 2R + 3L and · 3R the translocated chromosomes, with the position of the centromere marked by the dot. When an exchange occurs in arm 2R, a dyad of composition 2L · 2R/2L · 2R + 3L is formed and the resulting competition for inclusion in the oötid leads to a more frequent recovery of 2L · 2R than 2L · 2R + 3L. The aneuploid gametes are of the following constitutions: 2L · 2R, · 3R (the more frequent) and 2L · 2R + 3L, 3L · 3R (the less frequent). Segregation at the first meiotic division is always of the centromere region of one chromosome arm from the centromere region of the homologous arm, giving rise to dyads of composition 2L · 2R/2L · 2R, 3L · 3R/3L · 3R and 2L · 2R + 3L/2L · 2R + 3L, · 3R/ · 3R from the no-exchange tetrads and to dyads of composition 2L · 2R/2L · 2R + 3L, 3L · 3R/3L · 3R and 2L · 2R + 3L/2L · 2R, · 3R/ · 3R when an exchange occurs between the centromere and the translocation. Thus, the observed frequency of aneuploid gametes may not necessarily result from a type of segregation at the first division different from that which produces orthoploid gametes, but may be interpreted as a simple consequence of exchange between the centromere and the translocation breakpoint. It should be pointed out that such an interpretation might be applicable for this type of unequal-armed translocation, but can hardly be considered an explanation for the production of aneuploid gametes by other types of translocations.

Although the production of structurally asymmetrical dyads in Drosophila is achieved only by the use of special genetic constitutions, there is, in some other animals, one chromosome pair for which this could be a regular feature, namely, the sex chromosomes. Where the female is the heterogametic sex, a difference in size of the sex chromosomes, with a homologous region between the centromere and the differential segment allowing exchange, could lead to an excess of gametes carrying one of the sex-chromosomes, with the extent of the excess depending upon the frequency of exchange and the degree of competition. However, where the male is heterogametic and all the products of meiosis are functional, non-randomness could have no role without the further assumptions that there is, in fact, an orientation of the second meiotic division spindle with respect to the first and that some physiological or genetic mechanism (as, for instance, chromatid bridges involving any one of the autosomes) operates to eliminate certain of the spermatids differentially. Evidence available at present does not support such a scheme (SCHULTZ and ST. LAWRENCE 1949) but without a more thorough knowledge of the chromosome behaviour in such cases it cannot be dismissed as a possibility to account for some measure of the observed discrepancies in the primary sex ratio.

SUMMARY

The frequency of recovery of ring chromosomes from attached X chromosomes heterozygous for an inversion is shown to be inconsistent with the

expectations based on a tetrad analysis; the cause appears to be non-random disjunction at the second meiotic division when two structurally dissimilar chromatids compete for inclusion in the functional egg nucleus. Experiments involving structurally dissimilar rod chromosomes confirm this effect and the bearing of this phenomenon on the interpretation of previously existing data, particularly that on segregation in translocation heterozygotes, is discussed. It is suggested that such non-random disjunction could be responsible to some extent for observed deviations from the expected 1 : 1 sex ratio in animals where sex is determined by a heteromorphic pair of chromosomes.

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ON THE INTERPRETATION OF THE DOSE-FREQUENCY CURVE IN RADIOGENETICS

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IN a recent paper with the above title by OPATOWSKI (1950) and in another by OPATOWSKI and CHRISTIANSEN (1950) it is asserted that the observed linear relationship between the number of lethal gene mutations induced in Drosophila and the X-ray dose producing them is compatible with a "multi-hit" theory of genetic damage. OPATOWSKI sets up a simple "multi-hit" theory and deduces from it certain formulae which agree excellently with the experimental results. His conclusions are vitiated, however, by an error in the mathematical argument and, when this is corrected, the experiments of SPENCER and STERN (1948) are seen to be incompatible with the multi-hit theory he sets up.

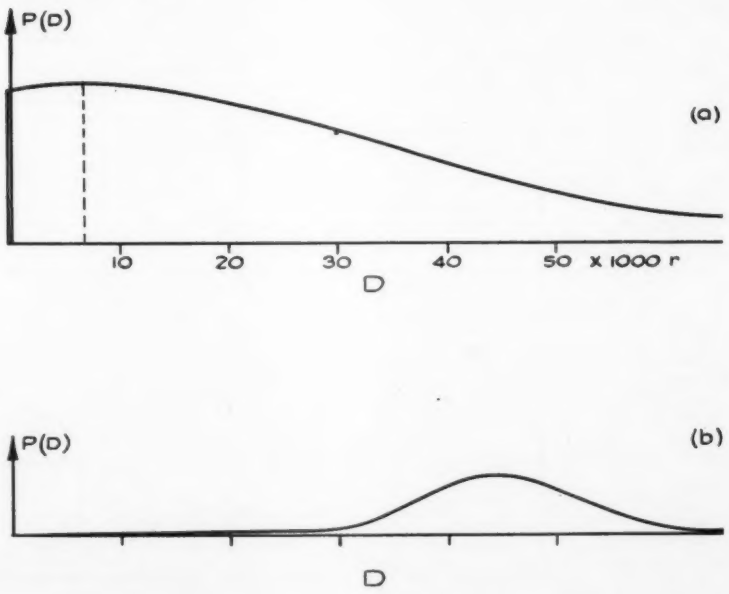

FIGURE 1.—A comparison of the distribution of D derived by OPATOWSKI (a), with the type of distribution predicted by statistical theory (b).

OPATOWSKI postulates that n events (ionizations) must occur within the gene (or in its near neighbourhood) in order to produce a mutation of the type considered, the i^{th} event requiring an increment in dose ΔD_i over the dose at which the $(i-1)^{th}$ event occurred. If the primary ionisations are distributed in random fashion throughout the irradiated volume the individual increments in dose ΔD_i can be taken to be positive random variables. The dose required to produce a mutation is then $D = \sum_{i=1}^{n} \Delta D_i$ and, if n is large, this sum, D, should tend to be normally distributed, irrespective of the parent frequency distribution of the ΔD_i. This is a well-known result in statistical theory (the Central Limit Theorem) but it is important to examine clearly its application to the present problem. OPATOWSKI fails to do this and arrives at a distribution which is contrary to the theorem. His method of selecting a suitable "normal" distribution is to take a normal curve truncated at an arbitrary point, $x = -S$, and choose the two parameters, h and M, of the normal curve and the extra parameter, S, thus introduced, in such a way as to fit SPENCER and STERN's data. The values chosen are $S = 42$ roentgens, $M = 6600$ roent-

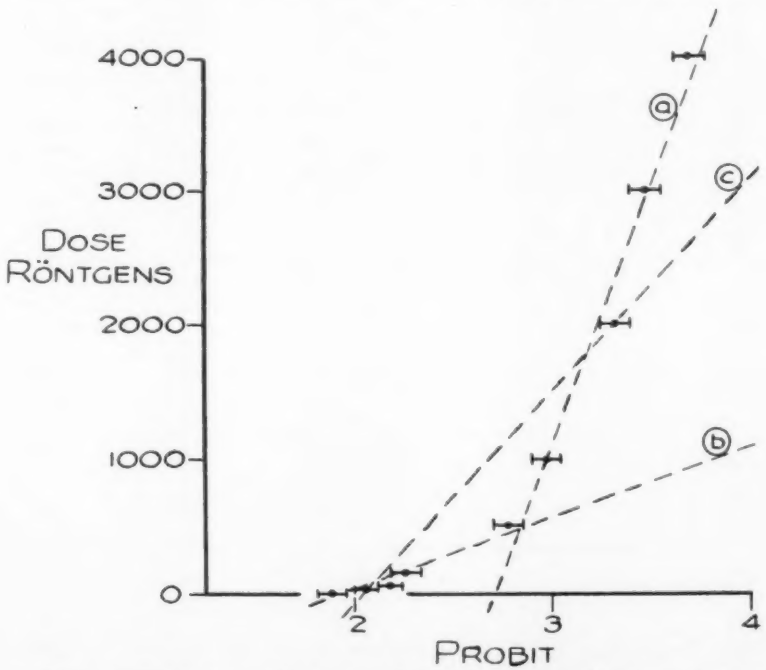

FIGURE 2.—Probit diagram for the data of SPENCER and STERN. Lines (a), (b) and (c) represent normal curves, but none can be found which fits all the data.

gens, $h = 2.5 \times 10^{-5}$ (roentgen)$^{-1}$. The shape of the distribution having these constants is shown in figure 1 a. It bears no resemblance to the distribution of the sum of n positive random variables drawn from any continuous frequency distribution. This sum should tend toward the normal form and should therefore look like figure 1 b for large values of n, where the ordinate at $D = 0$ of the best fitting normal curve is very small indeed.

The most convenient way of testing whether SPENCER and STERN's data can be fitted by a normal curve is the method of probits (FINNEY 1947). In figure 2, this test is applied to their data and it can be seen that the departure from linearity in the probit diagram is clearly significant. No normal curve can be found, therefore, which fits the data over the whole range. The limits of error shown in this figure are the fiducial limits for a fiducial probability $P = 0.95$, and are those quoted by SPENCER and STERN (1950, table 3).

The semilogarithmic coordinate system chosen by SPENCER and STERN and also by OPATOWSKI to represent the results does not demonstrate very clearly the high accuracy of the experimental data for low doses. It is better to use double logarithmic scale, as in figure 3. Moreover, since it appears that the induced mutation rate is linear with respect to dose, the spontaneous rate

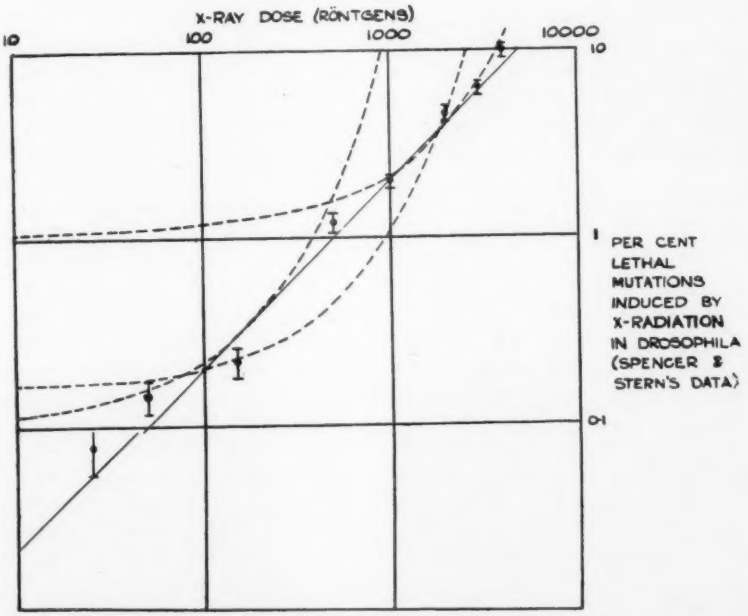

FIGURE 3.—Double logarithmic plot of SPENCER and STERN's data. The three dotted curves correspond to the normal distributions (a), (b) and (c) of Figure 2.

should simply be subtracted from each of the observed total mutation rates to obtain the induced rate, the standard error being increased accordingly. SPENCER and STERN's data have been plotted in this way in figure 3 and they do not appear to depart in any systematic way from a straight line, although a χ^2 test gives $P = 0.015$, which is not very convincing proof of linearity. The main contribution to the high value of χ^2 comes from the points at 150 and 4000 roentgens. Cumulative normal curves corresponding to the lines a, b and c in figure 2 are also drawn and it is quite clear that no normal curve fits the data over the whole range.

Summing up, we conclude, contrary to OPATOWSKI's view, that the experiments of SPENCER and STERN are not consistent with a multi-hit theory of genetic damage.

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EXPERIMENTS ON CHROMOSOMAL VARIABILITY IN DROSOPHILA ROBUSTA[1]

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MOST species of Drosophila so far studied in this respect are polymorphic for gene arrangements in their chromosomes. In *D. pseudoobscura, D. persimilis,* and *D. funebris,* this polymorphism is adaptive (DOBZHANSKY 1943, 1947, 1948a, 1948b, 1948c; DOBZHANSKY and EPLING 1944; DUBININ and TINIAKOV 1945, 1946a, 1946b; SPIESS 1950). The adaptive nature of the chromosomal polymorphism is shown by three types of evidence. (1) The frequencies of the gene arrangements often show clear geographic and altitudinal gradients. (2) Cyclic seasonal changes in relative frequencies of gene arrangements are observed in some localities. (3) Experiments on the behavior of chromosomes with different gene arrangements in artificial populations in the laboratory show that inversion heterozygotes possess, as a rule, a much higher fitness than do the corresponding inversion homozygotes. CARSON and STALKER (1947, 1949) STALKER and CARSON (1948) have discovered chromosomal polymorphism also in *D. robusta* Sturtevant, a species common in forested regions of the eastern United States. In addition to the usual paracentric inversions, *D. robusta* populations contain also at least two pericentric and two cytologically apparently terminal ones. Geographic and altitudinal clines have been demonstrated for certain gene arrangements, but seasonal cyclic variations are small or absent in the populations living near St. Louis, Missouri.

The work reported in the present paper is a study of natural and experimental populations of *D. robusta* in the New York City area. The evidence shows that the chromosomal polymorphism in this species is also adaptive, inversion heterozygotes possessing higher adaptive values than do the homozygotes. The work was started during the summer of 1947. Preliminary collections showed that the gene arrangements 2L, 2L–1 and 2L–3 in the left arm of the second chromosome would be especially suitable for experimental study. Experimental populations with different combinations of the gene arrangements were created, and the ensuing changes in the relative frequencies of the chromosomal types in these populations were recorded by means of periodic sampling. In the spring and fall of 1948 and in the spring of 1949 systematic collecting was made in a single locality in New Jersey to detect possible seasonal variations in the incidence of the gene arrangements and to obtain a larger number of strains for further experimental populations.

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MATERIALS AND METHODS

Collections of Drosophila for this study were made in 1947 in deciduous woods on the outskirts of Bronx County, New York (3); in Tibbetts Brook Park, Westchester County, New York (10); near Fort Lee, Bergen County, New Jersey (6); in the Mariners' Harbor section of Richmond County, New York (11); at Cold Spring Harbor, Suffolk County, New York (2); and at Tenafly, Bergen County, New Jersey (1). In the parentheses are the number of strains developed from each place. Mass cultures were developed from each collected female, and the salivary glands of twenty to thirty larvae from each culture were smeared in aceto-orcein for a qualitative analysis.

The quantitative collections were made at Englewood Cliffs, Bergen County, New Jersey, in an area about 30×120 feet. The traps (Mason jars) were maintained throughout the study by fastening them to branches with wire. Collected females were placed individually in culture bottles; after five days they were transferred to fresh food. If larvae appeared in either bottle a salivary smear was made from a single female larva, and the recorded arrangements contributed to an "egg sample" (following the terminology of DOBZHANSKY and LEVENE 1948). If no larvae appeared, the female, presumably uninseminated, was mated to males homozygous for all chromosomal arms. Examination of seven to nine larvae from this cross determined the chromosomal constitution of the collected fly; the sum of these results may be called an "adult female sample." The corresponding "adult male sample" was obtained from matings of collected males and virgin stock females, care being taken in each instance to examine the salivary glands of at least one *female* larva. The homozygous stock used for most of the determinations, derived from a Westchester County strain, was homozygous for arrangements XL, XR, 2L, 2R, 3L and 3R.

The population cages used for *D. pseudoobscura* by WRIGHT and DOBZHANSKY (1946) are applicable also for *D. robusta*. The approximate duration of the egg to egg cycle in the cages was 35 days at 25°C, 50 days at 21°C, and 68 days at 15½°C.

The earliest populations were taken from pair matings out of the mass cultures as described by WRIGHT and DOBZHANSKY (1946). The offspring of similar homozygotes from the collections, as well as from these matings, were retained as homozygous stocks for use in subsequent cages. In addition, the F_1 or F_2 from collected flies were used in the makeup of later populations studying 2L vs. 2L–1 or 2L vs. 2L–3 when this would introduce no other arrangements of this arm.

In New York experiments at 15½°C were kept in a constant temperature room. The relative humidity varied considerably throughout the year, the range of monthly means being from 62.7 ± 0.8 percent (March, 1949) to 83.5 ± 0.9 percent (July, 1948). Some experiments at 25°C were also run in a constant temperature room. The monthly means of relative humidity were between 65.8 ± 0.4 percent (January, 1949) and 76.5 ± 0.7 percent (July, 1949). For the other experiments incubators were used. In one set at 25°C

the monthly means of relative humidity ranged from 47.2 ± 0.3 percent (April, 1949) to 59.2 ± 0.7 percent (June, 1949). In another 25° incubator the monthly means varied from 89.9 ± 0.5 percent in March, 1949, to 92.0 ± 0.2 percent in August, 1949. One experiment was kept in a 21° incubator at generally close to 70 percent humidity. On September 8, 1949, a number of the experiments were moved to Virginia Polytechnic Institute, Blacksburg, Virginia, and continued in incubators there.

Because of the long life cycle of *D. robusta* a considerable problem in these experiments was the prevention of mite infections and contaminations with foreign Drosophila species. Benzyl benzoate described by WALLACE (1948) and a 5 percent dust of the miticide " DMC " and pyrophyllite (DAHM and BAUER 1949) in the cups with growing larvae were used to control the mites. (Technical compound " DMC " obtained through the courtesy of the Sherwin-Williams Company, Cleveland, Ohio, and pyrophyllite from the R. T. Vanderbilt Company, New York, New York.)

CHROMOSOMAL POLYMORPHISM IN NATURAL POPULATIONS

Data concerning the frequencies of the gene arrangements at Englewood Cliffs, New Jersey, are summarized in table 1. The fifteen gene arrangements found in more than one locality by CARSON and STALKER (1947) are all represented. The relative frequencies of the gene arrangements in this population are generally similar to their northern populations. Arrangements that show most obvious increases from south to north, XL–1, 2R, and 3R, show at Englewood Cliffs the highest frequencies yet recorded for these arrangements; correspondingly, XL, 2R–1, and 3R–1 show their lowest recorded frequencies. Another inversion more common in northern than in southern populations, the pericentric inversion, 2L–3, has here almost as high a frequency as in Iowa. Arrangements common in southern United States, such as XL–2 and 2L–2, are comparatively rare in Englewood Cliffs.

Comparison of the arrangement frequencies in spring and fall samples discloses no significant differences. The differences within the 1948 collections are probably attributable to sampling errors; even were they real, the spring, 1949, data show that the changes are not cyclic.

An autumnal virginal diapause similar to that described by CARSON and STALKER (1948) was found at Englewood Cliffs; all except the first two out of 84 mature-appearing females collected in the fall of 1948 were uninseminated in nature.

Zygotic frequencies in the egg sample, adult male sample, adult female sample, and combined adult sample (data kept on file to minimize publication costs) were studied by means of LEVENE's t-test (DOBZHANSKY and LEVENE 1948). In no instance is there a significant difference, at the 5 percent level using both tails of the curve, between the number of heterozygotes and homozygotes observed and the number expected in each sample by HARDY's formula. The adult female sample does show a large excess of heterozygotes for the X-left arrangements but this is probably not significant ($P = 0.088$). In

TABLE 1

Gene arrangements and their frequencies (in percent) in D. robusta collected at Englewood Cliffs, New Jersey, May 6, 1948–June 8, 1949.

	n_X	XL	XL-1	XL-2	XR	XR-1	XR-2	n_A	2L	2L-1	2L-2	2L-3	2R	2R-1	3R	3R-1
May 6–June 14, 1948	32	34.4	65.6	0.0	90.6	0.0	9.4	44	31.8	27.3	2.3	38.6	100.0	0.0	100.0	0.0
March 28–June 8, 1949	131	42.0	56.5	1.5	87.0	0.0	13.0	178	46.1	28.6	3.4	21.9	96.3	3.4	98.3	1.7
Both springs	163	40.5	58.3	1.2	87.7	0.0	12.3	222	43.2	28.4	3.2	25.2	97.3	2.7	98.6	1.4
August 24–October 3, 1948	236	39.1	60.2	0.4	88.1	0.4	11.4	323	41.2	28.2	5.9	24.8	98.8	1.2	96.9	3.1
Total	399	39.8	59.4	0.8	88.0	0.3	11.8	545	42.0	28.3	4.8	25.0	98.2	1.8	97.6	2.4

most other cases "t" is positive, indicating an excess of homozygotes; the deviation of this type is greatest for the combined adult sample of second-left arrangements (P = 0.102). In unpublished data CARSON and STALKER found more significant excesses of homozygotes. Natural populations of *D. pseudoobscura* have an excess of inversion heterozygotes in adult male samples (DOBZHANSKY and LEVENE 1948).

Table 2 shows gametic frequencies in adult samples, by season. Surprising differences are disclosed between the gene arrangements carried by males and females in the fall, 1948, collection. The females seem to have a significantly higher frequency of XL and 2L and a lower frequency of XL–1 and 2L–3 than do the males; the excess of 2L is highly significant (P less than 0.01). The data suggest that selection for the carriers of these arrangements is not equal in the sexes. The data suggest also that these sex differences show seasonal variation; for example, males seem to carry less 2L than females in the fall but more 2L in the spring. The data were tested for seasonal and sexual differences simultaneously by comparing the frequencies of the ar-

TABLE 2

Seasonal comparison of gametic frequencies (in percent) of XL and 2L arrangements in adult samples.

	n_X	XL	XL-1	XL-2	n_A	2L	2L-1	2L-2	2L-3
Fall males	87	31.0	69.0	0.0	175	34.3	29.7	6.9	29.1
Fall females	144	44.4	54.9	0.7	144	50.0	25.7	4.9	19.4
Spring males	61	41.0	57.4	1.6	118	44.9	28.0	3.4	23.7
Spring females	20	45.0	55.0	0.0	20	25.0	30.0	10.0	35.0
Total males	148	35.1	64.2	0.7	293	38.6	29.0	5.5	27.0
Total females	164	44.5	54.9	0.6	164	47.0	26.2	5.5	21.3

rangements in spring males, spring females, fall males and fall females, against the frequencies expected if neither season nor sex had any effect. No significant differences appeared between the carriers of X-left arrangements, 2L–1, and 2L–3, but males and females apparently do show a significant seasonal difference in their frequencies of the arrangement 2L (P slightly more than 0.01 for 3 degrees of freedom). A serious limit to the validity of this test is the rarity of virgin females in the spring collections.

EXPERIMENTAL POPULATIONS

Table 3 contains data describing the initial compositions of the population cages in this study. Experiments of DOBZHANSKY, HOLZ, and SPASSKY (1942) demonstrated the presence of many recessive deleterious mutants in chromosomes of *D. pseudoobscura* derived from natural populations. Presumably this is no less true for *D. robusta;* hence, an attempt was made to ensure heterogeneity of gene contents of the chromosomes used by including a minimum of five different strains of each gene arrangement. The number of strains used, listed in the table as "number of kinds of chromosomes," repre-

sents the minimal number of kinds of chromosomes, since the parental flies of a strain may contribute more than a single chromosome with the same gene arrangements. Populations 1 and 2 were derived from the same pair matings, hence from identical strains; with two exceptions the same was true of 3 and 4. In cages 1–16, initial populations contained equal numbers of males and females.

The experimental results are given as gametic frequencies, the bulkier zygotic data being kept on file. Unless otherwise noted, the chromosome number per sample is 300. The sample of the parental population, taken about two weeks after the experiment began, was called "control" sample. The

TABLE 3

Composition of the initial populations in 21 population cages.

Experiment number	Date begun	Temp. °C.	Gametic frequencies			Number of kinds of chromosomes			Number of flies
			2L	2L-1	2L-3	2L	2L-1	2L-3	
1	March 8, 1948	25	25	75	....	16	16	....	1000
2	March 18, 1948	15½	25	75	....	16	16	....	1000
3	August 30, 1948	25	78	22	....	14	11	....	518
5	November 12, 1948	15½	90	10	....	18	9	....	1000
6	November 12, 1948	15½	76	....	24	22	....	20	1000
7	November 17, 1948	15½	....	80	20	....	12	5	638
8	November 19, 1948	25	82	....	18	26	....	17	1110
9	December 1, 1948	25	....	85	15	....	13	6	1000
10	December 22, 1948	15½	....	20	80	....	13	7	1000
11	January 5, 1949	15½	20	....	80	14	....	19	1000
13	January 18, 1949	25	20	....	80	20	....	21	1000
14	January 25, 1949	25	....	20	80	....	17	9	1000
4	September 23, 1948	25	81	19	...	12	12	...	878
12	January 10, 1949	25	10	90	....	23	11	..	2000
15	May 13, 1949	25	....	60	40	....	11	7	1000
1A	January 8, 1949	25	22	78	....	16	16	..	?
2A	April 1, 1949	21	30	70	....	16	16	...	?
9A	May 27, 1949	25	....	80	20	....	13	6	?
12A	June 1, 1949	25	11	89	....	23	11	....	4562
3A	November 14, 1949	25	33	67	...	14	11	....	152
16	November 30, 1949	25	50	...	50	11	....	6	1000

sample taken after the first generation hatched was called "F_1." Since the intervals between subsequent samples were equal to the generation time, they were analogously labelled; no implication that the sampled population consisted wholly of that generation is intended.

A. *Populations with 2L and 2L–1*

Table 4 shows the results of two experiments with the arrangements 2L and 2L–1 maintained at about 15½°C. The first of these, cage 2, apparently reached an equilibrium at about 30 percent 2L and 70 percent 2L–1. For calculation of the adaptive values of the gene arrangements in this environment, cage 5, where changes continued for several generations, is more suitable.

TABLE 4

Samples from experimental populations of 2L vs. 2L-1 at 15½° and 21° C. (2 and 5 at 15½°, 2A at 21°).

Sample	Cage 2		Cage 5		Cage 2A	
	2L	2L-1	2L	2L-1	2L	2L-1
Initial	25.0	75.0	90.0	10.0	30.0	70.0
Control	22.0	78.0	90.7	9.3	....	....
F_1	25.3	74.7	62.7	37.3	....	....
F_2	31.0	69.0	54.0	46.0	19.7	80.3
F_3	32.3	67.7	42.5	57.5*	18.7	81.3
F_4	30.0	70.0	55.3	44.7†	....	...
F_5	....	..	49.7	50.3	....	...
F_6	....	....	48.3	51.7	...	..
F_7	....	...	51.0	49.0	..	..
F_8	....	...	46.3	53.7	...	..
F_9	....	...	50.3	49.7	..	..

*n = 200.
†first sample in Virginia.

Using the method of iteration and the underlying assumptions described in WRIGHT and DOBZHANSKY (1946) gives the following results:

Genotype	W		
2L/2L	0.060	s = 0.940	
2L/2L-1	1.000		$\hat{q} = 0.501$
2L-1/2L-1	0.055	t = 0.945	

W represents the relative adaptive value, taking that of the heterozygote to be 1.00, s, the selective disadvantage of the 2L homozygotes, and t, the selective disadvantage of the 2L–1 homozygotes; "$\hat{q}$" is the equilibrium frequency of 2L expected from these selection coefficients. Here both homozygotes appear to be nearly lethal relative to the heterozygotes. The indicated equilibrium point is apparently the one obtained. With the exception of the small F_3 sample, the data fit closely the curve determined by these coefficients.

When cage 2 had apparently ceased changing, it was transferred to 21°C and 70 percent humidity, and renamed cage 2A. Table 4 shows that a change occurred at the new temperature in the reverse direction from the change in cage 2. Unfortunately, the experiment had to be discontinued before the point could be determined. The small change observed in the third generation makes it likely that an equilibrium was being approached, if not already reached.

At 25° these gene arrangements were studied in three groups of experiments (table 5). Cages 1 and 12A were kept in a constant temperature room where the average humidity varied from 65 to 76 percent. In No. 1 the fall of 2L frequency from the control of 28 percent during the first three generations certainly seemed to be produced by selection, but beyond this the experiment gives no clear results. Humidity in the room decreased at this time, and the erratic changes observed may be due to selection, the sign of which

TABLE 5

Samples from 2L vs. 2L-1 experimental populations at 25° C.

Sample	65–76% Humidity				45–60% Humidity				89–92% Humidity					
	Cage 1		Cage 12A		Cage 3		Cage 1A		Cage 4		Cage 12		Cage 3A	
	2L	2L-1	2L	2L-1	2L	2L-1	2L	2L-1	2L	2L-1	2L	2L-1	2L	2L-1
Initial	25.0	75.0	11.0	89.0	77.5	22.5	22.0	78.0	81.3	18.7	10.0	90.0	33.0	67.0
Control	27.7	72.3	...	....	77.7	22.3	20.0	80.0	77.3	22.7	12.3	87.7	42.0	58.0
F_1	23.0	77.0	13.3	86.7	64.7	35.3	16.0	84.0	72.7	27.3	13.0	87.0	41.7	58.3
F_2	...	....	13.7	86.3	53.7	46.3	8.3	91.7	54.3	45.7	10.7	89.3	33.7	66.3
F_3	13.3	86.7	13.7	86.3	50.0	50.0	9.0	91.0	50.7	49.3	11.0	89.0	40.7	59.3
F_4	14.7	85.3			36.3	63.7	8.3	91.7	45.3	54.7			31.0	69.0
F_5	17.3	82.7			32.7	67.3	7.0	93.0	44.3	55.7			33.7	66.3
F_6	22.7	77.3			41.0	59.0	7.7	92.3	42.3	57.7				
F_7	22.0	78.0			24.7	75.3			35.7	64.3				
F_8	20.0	80.0*			34.7	65.3			33.3	66.7				
F_9					34.3	65.7			..	..				
F_{10}					32.3	67.7†			42.3	57.7†				
F_{11}					33.0	67.0			31.7	68.3				
F_{12}									29.0	71.0				
F_{13}									38.7	61.3				
F_{14}									34.0	66.0				
F_{15}									40.7	59.3				
F_{16}									36.7	63.3				
F_{17}									35.7	64.3				

*Part of this generation as No. 1A.
† First sample in Virginia.

changed with the changes in humidity. Evidence for this comes from the equilibrium of about 20–22 percent which 2L apparently attained when the environment became stabilized. An alternative hypothesis would assume an equilibrium between 15 and 20 percent 2L which was obscured by sampling errors and genetic drift. Responsibility of sampling errors is contradicted by the zygotic data for F_3–F_8 which show greater differences than might be expected from sampling variation (P about 0.01 for twelve degrees of freedom). In cage No. 12A no significant changes were detected during two and two-thirds generations. The seeming inability of the 2L frequency to increase beyond 13.7 percent may, considering sample errors, support the hypothesis of an equilibrium frequency of 15–20 percent 2L in this environment.

Cages 3 and 1A were kept at 25° but at a lower humidity. Cage 1A was a continuation of experiment 1. Once in the new conditions (see table 5) the frequency of 2L fell again, apparently reaching an equilibrium around 8 percent 2L. This result seems very unlikely on a basis of genetic drift alone. Even better evidence for natural selection in an experimental population comes from cage 3. Starting with a high frequency of 2L and a low one of 2L–1, this population showed clearly directional changes for five generations, the fall in 2L and rise in 2L–1 apparently ceasing after a frequency of 33 percent 2L and 67 percent 2L–1 had been reached. After that, a few irregular changes occurred, and the cage was discontinued after the eleventh generation. The population was always small; thus, it contained 152 flies in F_{11} generation. Calculation of adaptive values using all the data for No. 3 gives the following results:

Genotype	W		
2L/2L	0.26	s = 0.74	
2L/2L-1	1.00		$\hat{q}_{2L} = 0.327$
2L-1/2L-1	0.64	t = 0.36	

The expected equilibrium proportion of 2L agrees with the one apparently attained. However, the inclusion of several generations of no apparent change has a considerable effect on the calculations. Thus, if the data used are restricted to the period until equilibrium seemed to be attained (F_5) the following picture of the selection coefficients emerges:

Genotype	W		
2L/2L	0.39	s = 0.61	
2L/2L-1	1.00		$\hat{q}_{2L} = 0.116$
2L-1/2L-1	0.92	t = 0.08	

The adaptive values of the heterozygotes and the 2L–1 homozygotes seem to have been more nearly similar during the period of rapid change than was indicated by the previous calculation. An equilibrium is again predicted. However, this equilibrium is very different from one apparently attained in this cage; in fact, it closely resembles the one attained in cage 1A as well as several results at this temperature at higher humidities (cages 12, 12A, and the

first part of cage 1). Below are the calculated expected frequencies of 2L on the basis of each set of adaptive values for comparison with the observed results:

Sample	Actual	Expected (First set)	Expected (Second set)
Control	.777	....	...
F_1	.647	.617	.651
F_2	.537	.504	.536
F_3	.500	.437	.447
F_4	.363	.397	.378
F_5	.327	.373	.330
F_6	.410	.357	.294
F_7	.247	.347	.266
F_8	.347	.341	.244
F_9	.343	.337	.226
F_{10}	.323	.334	.212
F_{11}	.330	.332	.200
Limit	..	.327	.116

As expected, the adaptive values indicating an equilibrium of 11.6 percent 2L (second set) fit the gametic data of the first five generations more closely than do the ones in the first set. If, then, this is the correct set for these generations, the discrepancies between observed and expected in the later data would mean that the adaptive values of the genotypes are different when the frequencies are about 33 percent 2L and 67 percent 2L–1 in this environment than they are at higher frequencies of 2L or lower ones of 2L–1. However, the calculations based on all the data (first set) also generally agree, within the commonly accepted limits of sampling error, with the observed frequencies during the period of rapid change. The only exception is the F_3 sample, which has $t = 2.20$, and this also is not highly significant ($P = 0.028$). Since it is also simpler, because no variation of the adaptive values need be postulated, the hypothesis of adaptive values leading to an equilibrium of 33 percent 2L is probably the preferred one.

Experiments 4, 12, and 3A were kept in the 25° incubator at about 90 percent humidity. These cages consistently contained large populations; for example, F_3 of cage 12 numbered 4562 individuals. Table 5 shows that the changes in No. 4 were of the same general nature as those in its sister cage, No. 3, but they were slower arriving at the equilibrium point. The calculated selection coefficients are:

Genotype	W		
2L/2L	0.35	$s = 0.65$	
2L/2L-1	1.00		$\hat{q}_{2L} = 0.350$
2L-1/2L-1	0.65	$t = 0.35$	

Cage 12, intended as a check on population 4, showed no significant changes. Experiment 3A determined whether the equilibrium attained in No. 3, of which it was a continuation, would change in higher humidity. The available data indicate no change, thus possibly duplicating the result in cage 4.

Because of the relative rarity of 2L–3 in the 1947 collections, experimental

populations involving this pericentric inversion were not begun until November, 1948. It was, therefore, not possible to carry out as many tests in different environments as was possible for 2L and 2L–1. It is hoped to study the adaptive properties of 2L–3 more fully later.

B. *Experiments with 2L and 2L–3*

The studies with 2L and 2L–3 are shown in table 6. At the lower temperature these consist of cages 6 and 11. Cage 11, which could be carried on for only two generations, showed no significant changes. No. 6 did show significant changes for a number of generations. These changes make it likely that these arrangements are responsive to natural selection in this environment and indicate that the data of cage 11 resulted from beginning the experiment at or near the equilibrium point. The relative adaptive values of the genotypes in experiment 6, based on the available data, are:

Genotype	W		
2L/2L	0.53	s = 0.47	
2L/2L-3	1.00		$\hat{q}_{2L} = 0.434$
2L-3/2L-3	0.64	t = 0.36	

The gametic frequencies expected from these adaptive values agree closely with those found in the samples. This applies to the period of rapid change as well as the later data, indicating that natural selection was governed by the same adaptive values throughout the range of frequencies covered by this experiment. Not explained, however, is the discrepancy between the equilibria for experiments No. 11 and No. 6.

At the higher temperature also, the cage begun with high frequency of 2L–3, No. 13, showed no significant directional change. An increase in frequency of 2L and decrease in 2L–3 did occur during the second and third generations, but the population reverted to its initial frequencies thereafter. This suggests these are equilibrium frequencies for 2L or 2L–3 at this temperature. The population begun with low frequency of 2L–3, in this case No. 8, showed steady, albeit slow, decreases in the frequency of 2L and increases in 2L–3, apparently due to natural selection. This experiment had to be discontinued before the final outcome could be determined. To see if the results in No. 13 could be duplicated, No. 16 was begun with 50 : 50 proportions of 2L and 2L–3. The control sample showed a few 2L–1 chromosomes had also been introduced. The main change observed is the rise in 2L–1, obscuring the relations of 2L and 2L–3.

These results indicate that the carriers of 2L and 2L–3 have different adaptive values, probably resulting in equilibria, at both temperatures, but the data do not show conclusively whether the equilibria differ at the different temperatures.

C. *Experiments involving 2L–1 and 2L–3*

The several experiments involving these overlapping inversions are shown in table 7. Cages 7 and 10 show no significant changes and apparently dem-

TABLE 6

Samples from experimental populations of 2L vs. 2L-3. Cages 6 and 11 at 15½° C., 13 and 16 at 25° C. and 45-60% humidity, 8 at 25° C. and 65-76% humidity.

Sample	Cage 6		Cage 11		Cage 8		Cage 13		Cage 16			
	2L	2L-3	2L	2L-3	2L	2L-3	2L	2L-3	2L	2L-3	2L-1	n†
Initial	76.2	23.8	20.0	80.0	82.0	18.0	20.0	80.0	50.0	50.0	....	....
Control	80.3	19.7	30.3	69.7	78.0	22.0	21.0	79.0	45.4	53.2	1.3	308
F_1	77.3	22.7	24.3	75.7	80.7	19.3	21.0	79.0	44.7	54.6	0.7	304
F_2	69.3	30.7	26.7	73.3*	78.3	21.7	26.0	74.0	44.6	53.5	1.9	312
F_3	63.3	36.7			74.3	25.7	32.0	68.0	40.7	52.3	7.0	300
F_4	52.7	47.3 §			73.7	26.3	22.0	78.0	....	....	....	....
F_5	54.0	46.0			70.7	29.3	21.7	78.3	40.0	51.7	8.3	300
F_6	50.7	49.3			70.7	29.3	....	....	43.3	44.7	12.0	300
F_7	51.0	49.0			60.3	39.7	....	....	41.0	41.3	17.7	300
F_8	47.3	52.7										
F_9	47.3	52.7										

* n = 270.
† applies to cage 16 only.
§ First sample in Virgina.

TABLE 7

Samples from experimental populations of 2L-1 vs. 2L-3. Cages 7 and 10 at 15½° C., 14 and 9A at 25° C., 45-60% humidity, 9 and 15 at 25° C. and 65-76% humidity.

Sample	Cage 7		Cage 10		Cage 14		Cage 9A		Cage 9		Cage 15	
	2L-1	2L-3	2L-1	2L-3	2L-1	2L-3	2L-1	2L-3	2L-1	2L-3	2L-1	2L-3
Initial	80.2	19.8	19.8	80.2	20.0	80.0	80.0	20.0	85.0	15.0	60.0	40.0
Control	72.7	27.3	24.3	75.7	30.3	69.7	82.0	18.0	83.3	16.7	67.3	32.7
F_1	78.7	21.3	30.3	69.7	48.0	52.0	84.3	15.7	76.0	24.0	63.7	36.3
F_2	75.1	24.9	22.0	78.0	63.0	37.0	....	....	79.0	21.0	69.7	30.3
F_3	79.3	30.7	24.4	75.6*	63.7	36.3	88.7	11.3$	80.0	20.0		
F_4					74.7	25.3	88.7	11.3	80.0	20.0		
F_5					75.0	25.0$	85.7	14.3	82.0	18.0**		
F_6					73.0	27.0	70.3	29.7				
F_7					72.7	27.3	70.0	30.0				
F_8					69.3	30.7	76.0	24.0†				
F_9					67.0	33.0	74.0	26.0				
F_{10}					61.0	39.0						
F_{11}					52.0	48.0						
F_{12}					....	....						
F_{13}					44.0	56.0						
F_{14}					49.7	50.3						
F_{15}					48.0	52.0†						
F_{16}					51.0	49.0						

* n = 250.
† n = 50.
$ First sample in Virginia; development and hatching of these generations occurred in New York.
** Part of this generation spent as Cage 9A.

onstrate adaptive equivalence of the two gene arrangements at the low temperature. The behavior is different at 25°. Here a significant change was found in the population, No. 14, begun with a high frequency of 2L–3 and a low frequency of 2L–1. Calculation of adaptive values for the genotypes in this population, based on the samples of generations that developed in New York (Control–F_5), gives the following results:

Genotype	W		
2L-3/2L-3	0.17	s = 0.83	
2L-1/2L-3	1.00		$\hat{q}_{2L-3} = 0.153$
2L-1/2L-1	0.84	t = 0.16	

Had no change of environment taken place, it is not inconceivable that this equilibrium might have been reached. However, it is apparent that in Virginia there was a new change leading to equilibrium around 50 percent of each arrangement. The environmental factors responsible for the new change are presently under investigation.

Population No. 9, started with a high frequency of 2L–1 and low one of 2L–3, showed no significant changes in five generations. Upon transfer to a lower humidity, however, as No. 9A, this population changed in a manner reminiscent of the transfer of population 1 to a lower humidity (cage 1A, table 7). The decrease in 2L–3 and increase in 2L–1 in 9A is similar to what is expected from the New York equilibrium indicated for this environment in cage 14. An opposite change seemed to occur in Virginia in the same direction, but not to the same extent, as the new change noted in cage 14.

Since significant changes were noted only at the low humidity, the possibility exists that at higher humidity at this temperature (as in cage 9), the carriers of these arrangements are adaptively neutral. Cage 15, run at the

TABLE 8

Population counts of experimental populations.

Cage	Approximate generation	Temp. °C.	Humidity	Cage Style	Total flies	Females %	Males %
5	F_2	15½	63	New*	524	75.4	24.6
7	F_3	15½	79	Old*	624	78.7	21.3
10	F_3	15½	79	Old	591	82.5	17.5
2A	F_3	21	68	New	440	70.0	30.0
14	F_1	25	53	Old	137	64.3	35.7
13	F_5	25	57	Old	217	56.7	43.3
3	F_{11}	25	55	New	152	63.2	36.8
9A	F_3	25	53	Old	403	74.4	25.6
16	P_1	25	50	Old	361	71.7	28.3
16	F_1	25	52	Old	393	74.6	25.4
8	F_7	25	73	Old	1563	64.4	35.6
15	F_2	25	73	New	1157	76.2	23.8
12A	F_3	25	73	New	807	74.8	25.2
12	F_3	25	92	New	4562	61.7	38.3

*explanation in text.

same humidity as No. 9, is an attempted test of this hypothesis. Its results are inconclusive.

Size of the populations

Table 8 shows the counts made of various experimental populations along with the approximate mean humidity during the previous generation period. Females are consistently more common than males. At the high temperature the order of magnitude of the total count seems directly related to the relative humidity. At similar humidities, it is greater at high than at low temperature. At similar temperatures and humidities, the newer cages, those with netting instead of glass on two windows, and, therefore, with more evaporation surface, tend to have the smaller populations.

DISCUSSION

The *Drosophila robusta* population of Englewood Cliffs, New Jersey, repeatedly sampled for the purposes of the present investigation (see table 1), shows no cyclic seasonal changes in chromosomal composition, being in this respect like the St. Louis population studied by CARSON and STALKER (1949). It may be noted that no seasonal changes are observed for the two gene arrangements, XL–1 and 2L–3, which show clear geographic and altitudinal gradients (CARSON and STALKER 1947; STALKER and CARSON 1948) but whose seasonal variation could not be determined in Missouri (CARSON and STALKER 1949). The available data confirm the conclusion of CARSON and STALKER that the adaptive function of the chromosomal polymorphism in *D. robusta* is not related to seasonal changes in the environment. In this respect the species resembles *D. persimilis* rather than *D. pseudoobscura* or *D. funebris* (DOBZHANSKY 1943, 1948a; DUBININ and TINIAKOV 1945).

Experiments on artificial populations nevertheless show that the gene arrangements in *D. robusta* are not adaptively neutral. The relative frequencies of the gene arrangements in experimental populations often show rapid and directional changes, clearly caused by operation of natural selection. In no case, however, do these changes lead to elimination of one and establishment of the competing gene arrangement. Instead, the relative frequencies of the gene arrangements reach equilibria, and the composition of the populations becomes more or less constant.

Equilibria may be produced by natural selection if heterozygotes are at all frequencies adaptively superior to the homozygotes. In the text the relative adaptive values of the genotypes were calculated on the basis of this hypothesis, and the results expected from the calculated adaptive values generally corresponded well with the results actually obtained. Implicit in these calculations were two further assumptions: that the adaptive values were constant throughout the range of arrangement frequencies and that they were identical in the sexes. There is some evidence, however, that these assumptions may represent an oversimplification. Thus, in several experiments (see especially No. 3, table 5) the equilibrium apparently attained may not be the

one expected from the adaptive values responsible for earlier changes. This may occur if at certain frequencies the genotypes take on adaptive values leading to a different equilibrium. Or, the homozygotes, adaptively inferior at most frequencies, may be adaptively equal to the heterozygotes (and to one another) in the vicinity of the frequencies where observed changes stopped, for the effect of adaptive equality of the genotypes is there indistinguishable from a stable equilibrium under natural selection. In either case, the postulated stability of the adaptive values would be contradicted. Also, it is probable that variations in adaptive values, possibly related to small environmental variations, were responsible for temporary changes noted in several experiments (especially in No. 1, table 5 and No. 13, table 6). The observation made in the text that, in nature, carriers of several arrangements are apparently subject to different selection pressures depending on their sex makes the assumption that a given genotype has the same adaptive value in both sexes in the laboratory populations also questionable.

An alternative hypothesis for production of adaptive equilibria is that heterozygotes be at almost all frequencies intermediate in adaptive value to the homozygotes but that the adaptive value of each homozygote fall as the frequency of the arrangement increases. There would then be a range of arrangement frequencies where adaptive values of the homozygotes were similar or equal, and here no selective changes would occur. The margins of the range would be points of equilibrium. This possibility is more difficult to attack and was not considered in the text. However, instances described in the text where different equilibria seemed to be attained by the same arrangements in the same environment may well depend on some such explanation. Although the issue is complicated by strain differences and other variables, in some of these cases there was indeed a range of frequencies not attained from either extreme (compare, for example, cages 2 and 5, table 4; cages 4 and 12, table 5; cages 6 and 11, table 6). This possibility is supported also by the excess of homozygotes observed in several adult samples in nature (discussed in the text). WRIGHT and DOBZHANSKY (1946) point out, however, that such an explanation may well apply in nature, where a genotype may be adaptively superior until so numerous that it must attempt to use ecological niches for which it is not well adapted, but it is more difficult to conceive of such a phenomenon in an experimental population cage, where the number of niches is limited. Determination of zygotic frequencies among adults in the cages, currently being investigated, should help clarify this problem. In one completed study, F_{16} adults of cage 4 (table 5) a significant excess of *heterozygotes* was found in females, the adult males fitting the HARDY ratio. (Details of this and further studies of adults will appear in a later paper.)

The observations on experimental populations lend support to the view that the relative frequencies of different gene arrangements in natural populations are not accidental, but are determined by natural selection. Of course, this applies to populations in apparent equilibrium as well as to populations with changing gene or inversion frequencies. However, the time of action of natu-

ral selection in the life cycle of this fly in nature is not clear. The egg sample, albeit small, indicates that at least the spring sample is panmictic and lacks differential viability at the egg stage. The various adult samples indicate no differential survival of heterozygotes over homozygotes, in either males or females, beyond the egg stage; the overall adult sample for left arm second chromosome arrangements even suggest superior survival value of the homozygotes. The remaining possibility is greater fecundity or sexual activity of the heterozygotes. This hypothesis calls for no deviations from HARDY-WEINBERG ratios, provided the adaptive differences between genotypes are similar in the sexes. Unfortunately, the hypothesis is exceedingly difficult to test by observations on natural populations; laboratory experiments are planned. DA CUNHA (1949) found an even more significant excess of adult homozygotes for the color types of *D. polymorpha* in natural populations, although there is evidence the heterozygotes are adaptively superior. WALLACE (1948) showed, in laboratory experiments, that superior fecundity of females heterozygous for the sex-ratio factor in *D. pseudoobscura* apparently maintains this factor in populations despite the adaptive weakness of females homozygous for it.

Females heterozygous for a pericentric inversion may possess inferior fecundity, because crossing-over within the inversion would generally lead to inviable duplication-deficiency gametes. This explains the rarity of pericentric inversions in natural populations (DOBZHANSKY 1941). MILLER (1939) found a pericentric inversion in natural populations of *D. algonquin*. Since most individuals with the pericentric inversion also carry an arrangement differing from it by two overlapping inversions, MILLER suggests this reduces the probability of crossing-over and hence erases the theoretical selective disadvantage. CARSON and STALKER (1947) point out also that single crossovers within the overlap lead to a bridge and fragment as in paracentrics. In *D. robusta* the pericentric inversion 2L–3 is sometimes found associated with arrangements differing from it by two overlapping inversions, 2L–1 or 2L–2, and these may "protect" it somewhat, but most commonly it is found with 2L, which differs from it by only a single inversion. The extent of crossing-over within the inversion is unknown, but the fact that about half the euchromatic area of the left arm of the second chromosome is involved indicates that it probably does occur in 2L/2L–3 females. Hence, the high frequency of 2L–3 here (table 1) and in Mt. Vernon, Iowa (CARSON and STALKER 1947), and its even higher frequency at elevations more than 1400 feet near Gatlinburg, Tennessee (STALKER and CARSON 1948), cannot be explained on the same basis as the *D. algonquin* case. The equilibria obtained or indicated in the experiments with 2L–3, especially those involving 2L, show that a more likely explanation for the persistence of this inversion in populations is the selective advantage conferred on individuals heterozygous for it by heterotic effects of genes on it with those on 2L and 2L–1. The data indicate that these effects probably do not depend on differential mortality in nature.

Under natural selection the carriers of genotypes best adapted to the en-

vironment leave more surviving progeny than do carriers of genotypes with lesser fitness. The fitness or "adaptive value" is, however, a composite of relative superiorities and inferiorities in a number of factors operating at all stages of the life cycle. One of the most obvious and most easily measured of these is differential viability or mortality, but it is apparent that fitness depends also on relative fecundity, sexual activity, longevity and other factors. Moreover, each of these general classes is further compounded of particular variables; thus, a genotype which produces a higher ratio of inviable gametes may yet possess superior fecundity by virtue of a large total of gametes produced.

Several experiments with gene arrangements 2L and 2L–1 attained similar equilibria although maintained at different temperatures; and at all temperatures used, 15½°, 21°, and 25°C, the equilibria were at 50 percent 2L or less. Similar results were obtained in the experiments with 2L and 2L–3, though these are not as extensive and the results not as definite. This indicates that heterosis involving these gene complexes is largely independent of temperature. Only 2L–1 with 2L–3 shows clear-cut temperature differences in equilibria, these gene arrangements being adaptively neutral or nearly so at 15½° but not at 25°. However, temperature is but one of many environmental variables to which the arrangements might be adapted. Several experiments attempted to study another variable, namely humidity. These showed that populations of *D. robusta* are indeed sensitive to this factor, for the size of the population was consistently greater at higher humidities than at lower humidities at the same temperature. Whether any of the gene arrangements studied are involved in adaptations to this factor is not so clear, however. When a population containing 2L and 2L–1 was kept at a lower humidity (see experiment 1A, table 7), the frequency of 2L fell from 20–22 percent to about 8 percent, 2L–1 showing a corresponding increase. However, several experiments with these arrangements, notably 1A and 12, attained similar results at very different humidities, while still other experiments, notably 4 and 12, attained very different equilibria at the same humidity. Repetition of these experiments with better control of humidity and strain differences should yield interesting results. Controlling humidity in desiccators, HEUTS (1947 and 1948) was able to demonstrate differential survival of pupae and adults of *D. pseudoobscura* carrying various gene arrangements. DOBZHANSKY and ZIMMERING have shown that selective responses by carriers of certain gene arrangements in *D. pseudoobscura* also depended on the nature of the yeast used (DOBZHANSKY 1948d and unpublished data). The recent discovery that the natural food of *D. robusta* consists of microorganisms that occur in slime fluxes (CARSON and STALKER 1950) may lead to experimental study of the food preference of carriers of different gene arrangements also in *D. robusta*.

In some of the experiments described in this paper the same arrangements were involved under similar conditions of temperature and humidity. In none of these duplicate experiments were quite the same equilibrium proportions

of the arrangements attained; in some, the differences were quite large. Contrastingly, replicate experiments with gene arrangements of *Drosophila pseudoobscura* usually result in attainment of uniform equilibria (WRIGHT and DOBZHANSKY 1946; DOBZHANSKY 1947 and 1948c). This difference between *D. pseudoobscura* and *D. robusta* may be related to the sensitivity of *D. robusta* to small environmental variations noted on numerous occasions during the course of the experiments. The genetic basis for this sensitivity, apparently intimately bound up with the chromosomal polymorphism, should prove an interesting subject for further study.

SUMMARY

A study is made of chromosomal polymorphism in natural and experimental populations of *Drosophila robusta*. The natural population of one locality in the New York City area proved to contain the fifteen widespread gene arrangements known in the species. The observed high frequencies of inversions XL–1, 2L–3, 2R, and 3R are characteristic for northern populations of the species. Cyclic seasonal variation in the frequencies of inversions has not been observed, but there is evidence of differential selection in the two sexes for several arrangements at different seasons. The adult population consists of heterozygotes and homozygotes in proportions consistent with the HARDY-WEINBERG equilibrium.

Twenty-one experimental populations containing mixtures in various proportions of two paracentric inversions, 2L and 2L–1, a pericentric inversion, 2L–3, in pairs, were placed in population cages and were kept at three temperatures, 15½°, 21°, and 25°C. At the highest temperature they were also kept at three different humidities. Most of these populations showed significant and directional changes in arrangement frequencies resulting from natural selection. These populations usually attained equilibria between the arrangements at which the competing gene arrangements were present with constant frequencies. This probably indicates that inversion heterozygotes are superior in adaptive value to homozygotes. In the experiments with 2L and 2L–1, and those with 2L and 2L–3, similar selective responses occurred at all temperatures used; experiments with 2L–1 and 2L–3 showed significant selective differences at 25° but not at 15½°C.

Results of the experimental populations indicate that the frequencies of the gene arrangements in nature are determined by natural selection, probably acting through adaptive superiority of inversion heterozygotes. Heterosis of this sort also accounts for the persistence of a pericentric inversion in these populations.

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ALLELES AT THE HISTOCOMPATIBILITY-2 LOCUS IN THE MOUSE AS DETERMINED BY TUMOR TRANSPLANTATION *

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PREVIOUSLY published data (GORER 1942; GORER, LYMAN and SNELL 1948) have shown that alleles at the histocompatibility-2 locus in the mouse have a dual effect, determining a blood group antigen and also susceptibility or resistance to certain transplantable tumors. The blood group is most easily demonstrated with sera from C57BL mice which have received one or more inoculations of A strain tumor 15091a. Such sera will agglutinate A strain red cells, indicating that the tumor and the red cells possess an antigen in common. In crosses between the A and the C57BL strains, the A erythrocyte antigen and susceptibility to A strain tumors are always found associated; hence they probably are determined by the same genetic locus. Three alleles have been recognized: *H-2*, characteristic of the A strain; *H-2^d*, found in the dba's; and *h-2*, heretofore regarded as characteristic of C57BL, CBA and P mice. The interpretation of *H-2^d* as a separate allele rests on three serological observations. Strain dba tumor P1534 will elicit antibodies in C57BL mice, but at a lower titer than those produced by 15091a. Strain dba red cells tested against anti-15091a serum give a slightly lower titer than do A red cells. Finally, this antiserum when absorbed with dba red cells still reacts in a dilution of 1 to 8 with A red cells.

Tests carried out by methods adopted to show linkage between histocompatibility genes and known marker genes (SNELL 1948) showed that *H-2* is closely linked with the locus for fused tail, *Fu*. In the experiments previously reported, though there were several animals that can have been either crossovers, or normal overlaps in the expression of fused, no unquestionable crossovers were obtained.

METHODS

This linkage has been used to detect new alleles at the *H-2* locus and to determine which allele is present in certain inbred strains. The cross used is as follows:

$$(M \times F) \times N$$

where M and N are any two inbred strains, and F is a strain carrying either *Fu*, or the closely linked gene, brachyury or *T* (DUNN and CASPARI 1945). The F parent is usually heterozygous (*Fu fu*), but only fused (or brachyury)

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F_1 are saved to cross to N. The offspring of this double cross are inoculated with an M strain tumor, and observed to determine which animals survive and which die. If fused tail shows linkage with resistance, in either the coupling or repulsion relationship, it can be inferred that strains M and N carry different alleles at the *H-2* locus. This is the basic theorem of the tests. If they carry the same allele (*e.g.*, M and N both $H\text{-}2^m fu/H\text{-}2^m fu$), mice of the test generation will have the formulae:

$$H\text{-}2^m fu/H\text{-}2^m fu \quad H\text{-}2^m Fu/H\text{-}2^m fu \quad h\text{-}2^l fu/H\text{-}2^m fu \quad h\text{-}2^l Fu/H\text{-}2^m fu$$

and, since susceptibility is dominant (LITTLE 1941), must either be all susceptible, or, if other histocompatibility loci are segregating, be part susceptible and part resistant but without linkage with fused.

It is also apparent that a linkage of fused tail with resistance proves that strains M and F carry different alleles at the *H-2* locus. If they carry the same allele, the F_1 will be $H\text{-}2^m fu/H\text{-}2^m Fu$, and linkage cannot be manifest.

The converse of these theorems is not true; the absence of linkage does not necessarily prove that either or both strain F and N carry the same allele as that carried by strain M. Thus strains F and N might carry alleles $h\text{-}2^l$ and $h\text{-}2^n$ such that $h\text{-}2^l/h\text{-}2^n = H\text{-}2^m/h\text{-}2^n$, in which case all inoculated mice would succumb (assuming no other factors for resistance). A known parallel is provided by the agouti locus in mice, where $a^t/A = A^w/A$, the light belly of a^t and A^w being dominant, though the non-agouti coat of a^t is recessive.

The transplantable tumors used were 15091a (mammary gland spindle cell carcinoma, A strain origin), P1534 (lymphoid leukemia, dba/2 origin), S621 (induced fibrosarcoma, B alb/c origin), C1498 (myeloid leukemia, C57BL origin) and S637 (induced anaplastic sarcoma, P origin). All are virulent, rapidly growing tumors. S621 and S637, the only two not previously described in the literature, were first transplanted in July 1948 and December 1948 respectively. A few tests were also made with C57BL mammary carcinoma E0771.

All inoculated animals were classified as + if they succumbed to the tumor, as – if they survived. There was temporary growth followed by regression in many of the latter group. A few of the positive animals survived much longer than the others. These were probably genetically resistant and are indicated as such by a footnote in table 1.

The fused tail gene shows some normal overlaps, particularly when the fused gene is inherited from the mother (REED 1937). To lessen this difficulty, male rather than female fused F_1 were selected as parents for the second cross. Also many of the normal tail resistant mice were tested genetically to determine if they were actually *Fu fu* instead of being crossovers. A number of such mice were found, of which three appear in one cross listed in table 1.

The inbred strains of mice used were: A, dba/2, B alb/c, C57BL/6, C57BL/10 (6 and 10 are closely related sublines) and P. The brachyury (*T*) line was kindly supplied by DR. DUNN. Two fused lines were used, the CA strain (indicated in table 1 by *Fu*) and fused-Columbia, a strain from DR. DUNN (indicated in table 1 by *Fu*/C).

RESULTS

The results are summarized in table 1. They fall into three general groups.

In the first group, of which the first two crosses in the table are typical, nearly all the normal tailed animals succumbed to the tumor while the *Fu* (or *T*) mice were resistant. Some of the normal tailed resistant (+−) animals may have been normal overlaps, others may be crossovers. There is close linkage, with the genes in the repulsion relationship. These crosses must have been segregating for at least two different histocompatibility-2 alleles.

In the second group, exemplified by the third cross in table 1, all inoculated mice, both fused and non-fused, succumbed. This establishes a presumption of, but does not conclusively prove, the identity of the allele carried by the first and either the second or third strains.

TABLE 1

Data showing linkage or absence of linkage between Fu (or T) and susceptibility (+) or resistance (−) to tumor transplants. With one exception noted in a footnote, mice were inoculated with a tumor native to the first strain listed for each cross.

Cross	++	+−	*Fu*+	*Fu*−	Conclusion
(A × *Fu*) × dba/2	12	1†	2(1§)	11	dba/2 is not *H-2*, is *H-2*^{d}
" × B alb/c	17	0	0	11	B alb/c is not *H-2*
(dba/2 × *T*) × B alb/c	10	0	12	0	B alb/c is *H-2*^{d}
(B alb/c × *Fu*) × dba/2	13	0	5	0	B alb/c is *H-2*^{d}
(A × *Fu*) × C57BL/10	5	1	0	8(3‡)	C57BL/10 is not *H-2*
(dba/2 × *T*) × C57BL/10	10	2	0	17	C57BL/10 is not *H-2*^{d}
(C57BL/6 × *Fu*/C) × A	10	0	1	10	C57BL/6 is *b-2*^{b}; *Fu*/C is not *b-2*^{b}
" × C57BL/6*	4	19	13	0	*Fu*/C is *H-2*
(A × *Fu*/C) × C57BL/10	28	0	15	1**	*Fu*/C is *H-2*
(A × *Fu*) × P	13	2	1	10	P is not *H-2*
(dba/2 × *Fu*) × P	35	3	5(2§)	33	P is not *H-2*^{d}
(C57BL/6 × *Fu*/C) × P	16(1§)	0	0	9	P is not *b-2*^{b}
(P × *Fu*/C) × C57BL/10	4	3	0	5	P is *b-2*^{b}

* Animals from this cross were inoculated with A strain tumor 15091a. In all other cases the tumor was native to the first strain named in the formula for the cross.

† Tested genetically; not a normal overlap.

‡ Tested normal overlaps.

§ Succumbed to the tumor, but probably genetically resistant on the basis of long survival.

** No palpable tumor growth at any time. Perhaps was not inoculated.

In the third group, consisting of the cross (C57BL/6 × *Fu*/C) × C57BL/6, inoculated with A strain tumor 15091a, there is linkage, but in the coupling instead of the repulsion relationship. The inoculated mice had an A strain great-grandparent, but no other A ancestors. Since all *Fu* mice succumbed to the A strain tumor, they probably carried the A strain allele, *H-2*.

DISCUSSION

The results prove the existence of four alleles at the histocompatibility-2 locus. These are: *H-2*, characteristic of strain A; *H-2*^{d}, characteristic of strain

dba/2 and B alb/c; *h-2*^{b}, characteristic of strains C57BL/10 and C57BL/6; and *h-2*^{p}, characteristic of strain P. The nature of the test is such that the identity of the allele in strains dba/2 and B alb/c cannot be regarded as beyond question. However, these stocks have been used in a number of crosses, some of them not listed in the table, and have always given identical results. Hence the presumption of identity at this locus is strong.

One of the unsolved questions in regard to histocompatibility genes is whether one allele at each locus is a complete recessive, or whether the situation is like the M, N blood types in man where each allele expresses itself in the heterozygote. The classical genetic theory of tumor transplantation (LITTLE 1941) assumes the first of these alternatives, and this alternative gives the best fit to the published data (SNELL 1948). However, because of the close similarity of histocompatibility genes to blood group genes the second alternative has considerable plausibility. The results here reported prove that, at least in the case of the histocompatibility-2 locus, the second alternative is correct. The allele *h-2*^{b}, previously treated as a complete recessive, actually behaves as a dominant in crosses where the tumor used is C1498 (C57BL origin). C1498 will grow if, and only if, *h-2*^{b} is present.

Another fact brought out in this investigation is the importance of this one locus in controlling susceptibility or resistance to old and virulent transplantable tumors. It is known that there are a dozen or more loci concerned with susceptibility and resistance to transplants (LITTLE 1941; SNELL 1948), but the tests demonstrating these were carried out with "young," recently derived tumors. It will be seen from table 1 that in all our crosses, involving a number of different strains and four transplantable tumors of diverse origin, there is no hint of any factor other than histocompatibility-2 causing resistance to transplants. The few exceptional animals are explainable as normal overlaps or crossovers (we have definite proof, to be published later, that crossovers between *Fu* and *H-2* do occur). Moreover, with the tumors included in table 1, only rarely do genetically resistant animals fail to inhibit tumor growth. In table 1, there are 4 probable exceptions out of a total of 377 mice. However, with other tumors exceptions are more common; C57BL tumor EO771 gave 6 probable exceptions out of 39 mice.

Additional stocks are being tested to determine what *H-2* allele they carry. Quite possibly these tests will reveal additional alleles.

As in every study of multiple alleles, there is a possibility that we are dealing with closely linked genes rather than true alleles.

SUMMARY

The histocompatibility-2 locus in the mouse determines susceptibility or resistance to certain transplantable tumors. By using the close linkage of this locus with the genes for fused tail (*Fu*) and brachyury (*T*), tests for new alleles have been carried out.

Four alleles have been identified: *H-2*, characteristic of strain A and necessary for the growth of strain A tumor 15091a; *H-2*^{d}, characteristic of strains

dba-2 and B alb/c and necessary for the growth of dba/2 tumor P1534 and B alb/c tumor S621; *h-2^b*, characteristic of strains C57BL/6 and C57BL/10 and necessary for the growth of C57BL tumor C1498; and *h-2^p*, characteristic of strain P and necessary for the growth of P tumor S637. All four alleles behave as dominants in crosses. There is no indication in the crosses here reported that any factor other than the particular *H-2* allele in question is necessary for the growth of these tumors.

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